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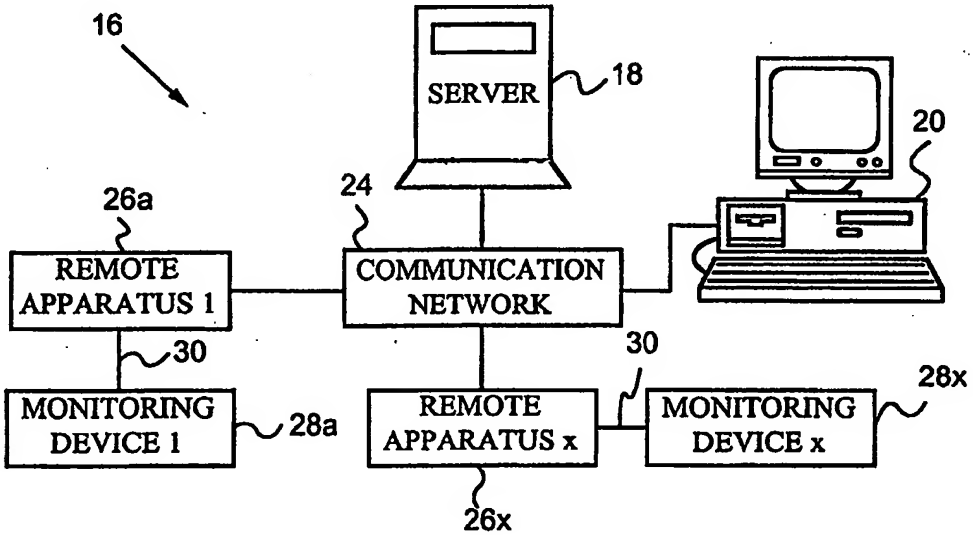

(57) Abstract

This invention is a networked system (16) for communicating information to a patient, and for remotely monitoring the patient.

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NETWORKED SYSTEM FOR INTERACTIVE COMMUNICATION AND REMOTE MONITORING OF DRUG DELIVERY

RELATED U.S. APPLICATION DATA

This application is a continuation-in-part of application Ser. No. 08/946,341, filed October 7, 1997, which is a continuation-in-part of application Ser. No. 08/847,009 filed April 30, 1997. The parent application claims priority from provisional application Ser. No. 60/041,746 filed March 28, 1997 and from provisional application Ser. No. 60/041,751 filed March 28, 1997. All of the above named applications are incorporated by reference herein.

FIELD OF THE INVENTION

The present invention relates generally to communication systems for remote monitoring of patients, and in particular to a networked system for remotely monitoring patients and for communicating information to the patients through the use of script programs. The invention further relates to a patient monitoring and drug delivery measurement system for measuring and electronically recording measurements of drug dose(s) administered to a patient. The invention also relates to injection syringes adapted for use with a dose measurement apparatus for electronically recording drug delivery measurements.

BACKGROUND OF THE INVENTION

Remote communication and monitoring systems

In the United States alone, over 100 million people have chronic health conditions, accounting for an estimated $700 billion in annual medical costs. In an effort to control these medical costs, many healthcare providers have initiated outpatient or home healthcare programs for their patients. The potential benefits of these programs are particularly great for chronically ill patients who must treat their diseases on a daily basis. However, the success of these programs is dependent upon the ability of the healthcare providers to monitor the patients remotely to avert medical problems before they become complicated and costly. Unfortunately, no convenient and cost effective monitoring system exists for those patients who have the greatest need for monitoring - the poor and the elderly.

Prior attempts to monitor patients remotely have included the use of personal computers and modems to establish communication between patients and healthcare providers. However, computers are too expensive to give away and the patients who already own computers are only a small fraction of the total population. Further, the patients who own computers are typically young, well educated, and have good healthcare coverage. Thus, these patients do not have the greatest unmet medical needs. The patients who have the greatest unmet medical needs are the poor and elderly who do not own computers or who are unfamiliar with their use.

Similar attempts to establish communication between patients and healthcare providers have included the use of the Internet and internet terminals. Although internet terminals are somewhat less costly than personal computers, they are still too expensive to give away to patients. Moreover, monthly on-line access charges are prohibitive for poor patients.

Other attempts to monitor patients remotely have included the use of medical monitoring devices with built-in modems. Examples of such monitoring devices include blood glucose meters, respiratory flow meters, and heart rate monitors. Unfortunately, these

monitoring devices are only designed to collect physiological data from the patients. They do not allow flexible and dynamic querying of the patients for other information, such as quality of life measures or psycho-social variables of illness. Nor do they allow for remote monitoring or recording of drug dose(s) administered to, or self-administered by, a patient.

Prior attempts to monitor patients remotely have also included the use of interactive telephone or video response systems. Such interactive systems are disclosed in U.S. Patents 5,390,238 issued to Kirk et al. on February 14, 1995, 5,434,611 issued to Tamura on July 18, 1995, and 5,441,047 issued to David et al. on August 15, 1995. One disadvantage of these systems is that they either require a patient to call in to a central facility to be monitored or require the central facility to call the patient according to a rigid monitoring schedule.

If the patients are required to call the central facility, only the compliant patients will actually call regularly to be monitored. Non-compliant patients will typically wait until an emergency situation develops before contacting their healthcare provider, thus defeating the purpose of the monitoring system. If the central facility calls each patient according to a monitoring schedule, it is intrusive to the patient's life, and resistance to the monitoring program grows over time.

Another disadvantage of these conventional interactive response systems is that they are prohibitively expensive for poor patients. Further, it is difficult to identify each patient uniquely using these systems. Moreover, these systems are generally incapable of collecting medical data from monitoring devices, such as blood glucose meters, respiratory flow meters, or heart rate monitors.

Remote monitoring of drug delivery

In recent years, the value of keeping electronic medical records in place of paper records has been widely recognized in the health care industry. The use of electronic medical records allows health care providers and patients to store, retrieve, and share medical information with considerably more ease and accuracy. The sharing of medical

information is particularly important in treatment programs involving the injection of insulin, human growth hormone, or other medications.

Typically, these injections are performed using disposable syringes. Unfortunately, no adequate apparatus exists that measures and electronically records dose information from a disposable syringe. As a result, the patient or health care worker performing the injection is burdened with the task of injecting the dose and then manually recording the dose amount in a logbook.

Because of the frequency of such injections, often several times a day for diabetics, it becomes difficult for a patient to keep accurate records. Indeed, studies have shown that a patient's own records and recollections are often incomplete and inaccurate. Additionally, a patient may intentionally cheat while making self-recorded entries in an attempt to create a logbook that will please his or her doctor. In the long-term this makes patient monitoring extremely difficult and jeopardizes the treatment program, possibly even endangering the patient's life.

Attempts have been made to develop electronic management systems for assisting patients in self-administered drug programs. For example, U.S. Patent 5,019,974 issued to Beckers describes a hand-held, microprocessor-based recorder that interfaces with a master computer. The patient enters therapy information into the recorder via a keyboard. The recorder includes a display for displaying treatment therapy guidelines to the patient. The recorder also has a blood glucose meter for recording the patient's blood glucose levels.

The recorder described by Beckers does not automatically measure and record dose information from a disposable syringe. After injecting a dose, the patient must manually enter the dose information into the recorder using switches or keys. Although this is an improvement over keeping written records on paper, the effectiveness of the drug program is still limited by the patient's recollections and recordings, which are unreliable.

Attempts have also been made to develop devices that deliver a predetermined dose of medication and record the dose amount. For example, U.S. Patent 5,176,502 issued to Sanderson et al. on January 5, 1993 describes a syringe pump for expelling a preset dose of medication from a syringe. The syringe pump includes a syringe retainer for holding the syringe and a driver for engaging the plunger of the syringe. An electric motor pushes the driver and plunger into the syringe barrel to expel the medication.

The syringe pump further includes a monitoring circuit for monitoring the motion of the driver during the delivery of the medication. The monitoring circuit includes a linear potentiometer having an electrically conductive strip of resistive material. The resistive material is positioned such that it engages an electrical contact of the driver. The position of the electrical contact on the resistive strip varies the voltage of the monitoring circuit, thus indicating the position of the plunger inside the barrel. A microprocessor receives voltage signals from the monitoring circuit and compares the voltage signals to preprogrammed signals to determine if the plunger displacement corresponds to correct displacement for delivering the preset dose. A control mechanism connected to the microprocessor regulates the driver's movement to ensure the preset dose of medication is delivered.

Although the syringe pump described by Sanderson does allow electronic recording of dose information, it is only designed to deliver medication directly into an intravenous line. It is not designed to inject a patient directly nor can it measure and record a dose from a syringe unless the syringe pump pushes the plunger. Consequently, the syringe pump is of little use to a health care worker who must inject a patient directly, or to an outpatient who must follow a self-injection treatment program.

Another device for injecting a preset dose of medication and for recording the injected dose is disclosed in U.S. Patent 4,950,246 issued to Muller on August 21, 1990. Muller describes a battery-operated injection pen having a pump rod driven by an electric motor. The electric motor is controlled by an electronic control unit that includes a

microprocessor with a memory for storing dose information. The injection pen further includes a sensor connected to the control unit for electrically determining the position of the pump rod, and thus the amount of medication injected.

Although the injection pen described by Muller measures and electronically records dose information, it has several disadvantages that have precluded its widespread use. The injection pen is an expensive device requiring complicated electronic equipment to deliver and record doses. Moreover, because the injection pen integrates a syringe and electronic recorder into one device, it is not disposable. The patient must use it repeatedly for each injection, even after the injection pen has been contaminated with blood. Consequently, the injection pen does not provide an inexpensive, convenient, or hygienic solution to patients wishing to measure and electronically record injected dose information.

U.S. Pat. 4,853,521 to Claeys presents a programmable, intelligent reader unit, which receives and records drug data using hand-held or fixed scanners. The scanners read bar codes in place on syringes, ampoules, flow meters, etc. In addition, this intelligent reader allows the user to weigh a syringe before and after injection to determine and record the administered amount of medicine. Dosage data logged in this manner can be displayed or printed out in the form of a record.

Operating the device described by Claeys requires many complicated steps of weighing syringes, scanning in bar codes, etc. The complexity of the required procedures, as well as the high cost of the apparatus, have precluded its widespread use. Additionally, the device cannot be easily carried by the user for recording doses while away from the health care facility or home. Thus, no inexpensive apparatus exists for determining and electronically recording dose information from a disposable syringe. Further, no such apparatus exists that is both simple in operation and easily carried by a user.

The following U.S. patents are incorporated by reference herein: U.S. patent No. 5,569,212; U.S. patent No. 5,628,309; U.S. patent No. 5,704,902; U.S. patent No. 5,720,733; U.S. patent No. 5,782,814; and U.S. patent No. 5,792,117. The following patent applications are also incorporated by reference herein: Serial No. 08/972,670; Serial No. 08/972,375; Serial No. 08/898,711; and Serial No. US97/12966.

OBJECTS AND ADVANTAGES OF THE INVENTION

In view of the above, it is an object of the present invention to provide a simple and inexpensive system for remotely monitoring patients and for communicating information to the patients. It is another object of the invention to provide a system, which allows flexible and dynamic querying of the patients. It is a further object of the invention to provide a system, which combines querying of patients with medical device monitoring in the same monitoring session. Another object of the invention is to provide a monitoring system which incurs lower communications charges than those incurred by conventional monitoring systems. A further object of the invention is to provide a monitoring system, which may be used at any time convenient for a patient.

It is a further object of the present invention to provide a system, which combines interactive communication between a healthcare provider and a patient at a remote location, with measurement and paperless recordation of drug dose(s) administered to the patient. It is a further object of the invention to provide an apparatus for inductively, capacitively, or optically determining, and for electronically recording, an injection dose delivered to a patient from a disposable syringe. It is another object of the invention to provide a drug dose measurement and patient monitoring apparatus that may be easily operated and carried by a user. A further object of the invention is to provide a patient monitoring and drug delivery measurement apparatus suited to diabetic patients, and to diabetes home care in particular. It is yet another object to provide an apparatus facilitating automated paperless data processing, from measurements performed by the patient to the recording at the clinic.

These and other objects and advantages will become more apparent after consideration of the ensuing description and the accompanying drawings.

SUMMARY OF THE INVENTION

The invention presents a networked system for remotely monitoring an individual, for communicating information to the individual, and for recording patient related data generated at a remote location. The system includes a server and a remote interface for entering in the server a set of queries to be answered by the individual. The server is preferably a worldwide web server and the remote interface is preferably a personal computer or network terminal connected to the web server via the Internet. The system also includes a remotely programmable apparatus for interacting with the individual. The remotely programmable apparatus is connected to the server via a communication network, preferably the Internet. The apparatus interacts with the individual in accordance with a script program received from the server. The server may also receive patient-related data from the remotely programmable apparatus via the communication network.

The server includes a script generator for generating the script program from the queries entered through the remote interface. The script program is executable by the remotely programmable apparatus to communicate the queries to the individual, to receive responses to the queries, and to transmit the responses from the remotely programmable apparatus to the server. The server also includes a database connected to the script generator for storing the script program and the responses to the queries.

The remotely programmable apparatus has a communication device, such as a modem, for receiving the script program from the server and for transmitting the responses and/or patient related data to the server. The remotely programmable apparatus also has a user interface for communicating the queries to the individual and for receiving the responses to the queries from the individual. In a preferred embodiment, the user interface includes a display for displaying the queries and user input buttons for entering the responses to the queries. In an alternative embodiment, the user interface includes a speech synthesizer for audibly communicating the queries and a speech recognizer for receiving spoken responses to the queries.

The remotely programmable apparatus also includes a memory for storing the script program and the responses to the queries. The remotely programmable apparatus further includes a microprocessor connected to the communication device, the user interface, and the memory. The microprocessor executes the script program to communicate the queries to the individual, to receive the responses to the queries, and to transmit the responses to the server through the communication network.

In one embodiment, the system also includes at least one monitoring device for producing measurements of a physiological condition of the individual and for transmitting the measurements to the apparatus.

According to another embodiment, a communication and monitoring system includes a patient monitoring and drug delivery measurement apparatus, in communication with the remotely programmable apparatus for providing measurements of drug dose(s) administered to, or self-administered by, a patient, wherein the communication and monitoring system includes: a server, a communications network, and a remotely programmable apparatus; and wherein the patient monitoring and drug delivery measurement apparatus includes; a dose measurement unit or element, a measuring device, a calibration memory, and a recording device.

A patient monitoring system of the invention may further include a device interface connected to the microprocessor for receiving physiological condition and/or drug delivery measurements from the drug delivery measurement apparatus. The measurements are stored in the memory and transmitted to the server with the responses to the queries. The server also preferably includes a report generator connected to the database for generating a report of the measurements and responses. The report is displayed on the remote interface.

According to one embodiment, the invention provides a system for non-invasively measuring and electronically recording a dose of a drug or medication delivered to a patient from a syringe. A currently preferred patient monitoring and drug delivery measurement system comprises apparatus for measuring and electronically recording a

dose of a drug administered to a patient from a syringe, wherein the dose delivered from the syringe may be measured inductively, capacitively, or optically. There now follows a summary description of three different embodiments of a drug dose measurement apparatus, each for use in conjunction with a patient monitoring system of the invention.

i) Optical dose measurement

In one embodiment of the invention, there is provided an apparatus for optically measuring a dose of a drug delivered to a patient, the apparatus including: a holding means for receiving and holding the syringe, a light source in optical communication with the syringe, an optical detector in optical communication with the syringe, and a recording means in electrical communication with the optical detector. An alignment means aligns the syringe barrel to the optical detector and/or the light source, when the syringe is in a measurement position.

The light source generates light incident on the syringe. An optical response of the syringe to the incident light is indicative of the liquid quantity within the syringe, and implicitly of the dose administered (or to be administered) from the syringe. The optical detector detects the optical response. The recording means records a dose datum indicative of the optical response and the dose. The dose can be computed from the optical response in conjunction with calibration or syringe parameter data, as will be described in detail herein below.

The incident light preferably includes wavelengths that are suitable for measuring typical plunger displacements (resolution on the order of 0.1 mm to 1 mm) and/or liquid quantities within the syringe (resolution on the order of 0.1 cm^3), and that interact minimally with elements of the syringe (e.g. the barrel) which do not vary with the quantity of liquid within the syringe. Such wavelengths are preferably, but generally need not be, in the visible or near-visible (infrared/ultraviolet) ranges. Preferably, the detector is suitable for detecting light within a range of wavelengths emitted by the light

source. Generally, the wavelength range emitted by the light source need not be identical to the wavelength range detected by the detector. In fact, the wavelength ranges need not even overlap, if the light detected by the detector results from absorption and re-emission by the syringe.

The light source and detector preferably comprise semiconductor emitting/detecting devices, but generally may include any device capable of emitting/detecting light of desired wavelengths. Such devices may include antennas or heat sensors. The recording means includes an electronic memory, preferably a digital memory unit.

The detector preferably includes a plurality of longitudinally spaced individual optical detecting elements coupled to the holding means and in optical communication with the syringe. The detecting elements detect an optical response pattern of the syringe, i.e. a spatial distribution of the syringe response. Dose data indicative of the optical response pattern is then recorded. The light source preferably includes plural longitudinally spaced light emitters. Each light emitter generates a light beam incident on the syringe. The optical response pattern is indicative of the interaction of the light beams with the syringe. Preferably, each of the light emitters is substantially aligned longitudinally with one of the detecting elements. If a control means in electrical communication with each of the light emitters is used to individually control each of the light emitters, a separate response pattern may be recorded for each emitter.

In an embodiment, which does not require an internal light source, the holding means encloses the syringe only on one side. The holding means does not completely enclose the syringe on the side opposite the detector, so as to allow external light to be incident on the syringe. The response pattern detected by the detector is then dependent on the interaction between the external light and the syringe.

In one embodiment, the syringe includes a response-enhancing element comprising an optical marking. The optical response of the syringe depends on the interaction of

incident light with the marking, and on the position of the marking. The position of the marking is indicative of the dose. The response-enhancing element may comprise a longitudinal element mechanically coupled to (e.g. on the surface of, or within) the syringe plunger. The longitudinal element is longitudinally marked by the marking. The marking may be a shape marking, or a color marking varying longitudinally in brightness and/or hue.

If the detector detects light transmitted or emitted by the syringe, the detector is situated opposite the light source relative to the syringe. If the detector detects light reflected by the syringe, the detector is situated adjacent the light source relative to the syringe (on the same side of the syringe).

A port connected to the recording means allows downloading dose data histories from the recording means to a host computer (storage and communications device). A display connected to the detector and/or recording means displays dose data including current doses and dose histories to the patient. Generally, the recording means may record any signal indicative of the optical response detected by the detector. For example, the recording means may record directly the optical response signal generated by the detector. Doses are then computed on a distinct computer after downloading of the recording means contents to the computer. Preferably, however, a computing means computes the dose data recorded by the recording means from the optical response by the detector.

Preferably, a housing encloses the light source, detector, recording means, and monitoring means. The holding means is mechanically coupled with the housing, and is preferably enclosed by the housing. The housing may be sufficiently compact to be hand-held and carried by a user, and may be battery-powered.

ii) Capacitance-based measurement

An apparatus for capacitively measuring and electronically recording a dose delivered from a syringe includes: a holder for receiving and holding a syringe in a measurement position; a capacitive element coupled to the holder and enclosing the syringe such that a capacitive response of the capacitive element is indicative of the dose when the syringe is in the measurement position; a measuring device connected to the capacitive element for measuring capacitive responses of the capacitive element; and a recording device connected to the measuring device for recording a dose datum indicative of the capacitive response and thus indicative of the dose.

Preferably, the holder includes a well, the well laterally enclosing the syringe when the syringe is in the measurement position. The capacitive element is then coupled to the well such that at least one electrode of the capacitive element laterally encloses the syringe when the syringe is in the measurement position. In one embodiment, the capacitive element is defined between the liquid held in the syringe and an external electrode situated outside the syringe. A needle contact coupled to the holder is then used to establish electrical communication between the measuring device and the liquid, through the syringe needle, when the syringe is in the measurement position. In another embodiment, the capacitive element is defined between first and second electrically conducting longitudinal plates coupled to the holder, electrically insulated from each other, and situated opposite each other relative to the syringe.

In yet another embodiment, the capacitive element is situated entirely within the syringe. Two coaxial cylindrical electrodes, one near the inside surface of the syringe barrel and the other near the outside surface of the syringe plunger, are connected to input and output terminals on the outside of the syringe barrel. The housing includes a contact field coupled to the outside of the housing. The contact field includes an input contact for contacting the input terminal, and an output contact for contacting the output terminal. The input and output contacts are connected to the measuring device.

A housing encloses the measuring and recording devices, and preferably encloses and magnetically shields the capacitive element. The holder is mechanically coupled to the housing. The housing is sufficiently compact to be hand-held and carried by a user. The capacitive element preferably consists of a single capacitor, and the capacitive response preferably includes the capacitance of the capacitor. In an alternative embodiment, the capacitive element includes plural longitudinally-spaced capacitors, and the capacitive response includes a capacitive response pattern.

iii) Inductance-based dose measurement

An apparatus for inductively measuring and electronically recording a dose delivered using a syringe includes: a holder for receiving and holding a syringe in a measurement position; an inductive element coupled to the holder and enclosing the syringe such that an inductive response of the inductive element is indicative of the dose when the syringe is in the measurement position; a measuring device connected to the inductive element for measuring inductive responses of the inductive element; and a recording device connected to the measuring device for recording a dose datum indicative of the inductive response and thus indicative of the dose.

Preferably, the holder includes a well laterally enclosing the syringe when the syringe is in the measurement position. The inductor is then coupled to the well such that the inductor laterally encloses the syringe when the syringe is in the measurement position. It is preferred that the syringe includes an inductance-enhancing element whose position relative to the syringe barrel is indicative of the dose, and whose position determines the inductive response of the inductive element. The inductance-enhancing element preferably includes a ferromagnetic or ferromagnetic longitudinal plunger element embedded in a plastic shell to form the syringe plunger. The inductance-enhancing element preferably includes a ferrite strip, but may comprise a ferromagnetic core filling the plunger cross-section almost entirely, or a series of longitudinally spaced, stacked

disks arranged within the plunger. Alternatively, a conventional syringe having a plunger consisting essentially of a plastic rod may be used.

A housing encloses the measuring and recording devices, and preferably encloses and magnetically shields the inductive element. The holder is mechanically coupled to the housing. The housing is sufficiently compact to be hand-held and carried by a user. The inductive element preferably consists of a single inductor, and the inductive response preferably includes the inductance of the inductor. In an alternative embodiment, the inductive element includes plural longitudinally spaced inductors, and the inductive response includes an inductive response pattern.

In one embodiment, the inductor is situated within the syringe barrel and is connected to input and output terminals on the outside of the syringe. The housing then includes a contact field coupled to the outside of the housing. The contact field includes an input contact for contacting the input terminal, and an output contact for contacting the output terminal.

For both inductive and capacitive measurement embodiments of the monitoring system, a port connected to the recording device may be used to download data stored in the recording device to an external storage or communication device, such as a host computer. Also connected to the recording device is a monitoring or testing device for testing a physical or physiological condition of the patient and for generating condition data representative of the physical or physiological condition. The recording device records the condition data. Preferably, the monitoring or testing device is a blood glucose meter and the physical or physiological condition is the patient's blood glucose level. A display connected to the measuring device is used to display recorded doses and blood glucose levels to the patient.

A computing device is connected to the recording device. The computing device computes dose data from dose measurement responses (capacitive, inductive, or optical)

and stored calibration data, for storage in the recording device. Dose data preferably includes administered doses. The calibration data, stored in a calibration memory device, is indicative of the correspondence between dose measurement responses and dose data for the particular syringe used by the patient. The calibration data is generated by measuring dose measurement responses for the entire range of potential liquid quantities in the syringe, and recording the correspondence between liquid quantities and dose measurement responses.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a block diagram of a networked system according to one embodiment of the invention.

Fig. 2 is a block diagram illustrating the interaction of the components of the system of Fig. 1.

Fig. 3 is a perspective view of a remotely programmable apparatus of the system of Fig. 1.

Fig. 4 is a block diagram illustrating the components of the remotely programmable apparatus of Fig. 3.

Fig. 5 is a script entry screen according to a preferred embodiment of the invention.

Fig. 6A is a listing of a sample script program according to a preferred embodiment of the invention.

Fig. 6B is a continuation of the listing of Fig. 6A.

Fig. 7 is a script assignment screen according to a preferred embodiment of the invention.

Fig. 8 is a sample query appearing on a display of the apparatus of Fig. 3.

Fig. 9 is a sample prompt appearing on the display of the apparatus of Fig. 3.

Fig. 10 is a sample report displayed on a workstation of the system of Fig. 1.

Fig. 11A is a flow chart illustrating the steps included in a monitoring application executed by the server of Fig. 1 according to a preferred embodiment of the invention.

Fig. 11B is a continuation of the flow chart of

Fig. **11A**.

Fig. **12A** is a flow chart illustrating the steps included in the script program of Figs. **6A-6B**.

Fig. **12B** is a continuation of the flow chart of Fig. **12A**.

Fig. **13** is a perspective view of a remotely programmable apparatus according to a second embodiment of the invention.

Fig. **14** is a sample prompt appearing on a display of the apparatus of Fig. **13**.

Fig. **15** is a block diagram illustrating the components of the apparatus of Fig. **13**.

Fig. **16** is a schematic block diagram illustrating the interaction of the server of Fig. **1** with the apparatus of Fig. **3** according to a third embodiment of the invention.

Fig. **17** is a first sample message appearing on the display of the apparatus of Fig. **3**.

Fig. **18** is a second sample message appearing on the display of the apparatus of Fig. **3**.

Fig. **19** is a script entry screen according to the third embodiment of the invention.

Fig. **20A** is a block diagram of a networked monitoring system including a remote patient monitoring and drug dose measurement apparatus, according to another embodiment of the invention.

Fig. **20B** is a high-level schematic diagram illustrating a patient monitoring and drug delivery measurement apparatus of the system of Fig. **20A**, according to the invention.

Fig. **21** is a perspective view of a drug delivery measurement apparatus according to another embodiment of the invention.

Fig. **22A** is a longitudinal sectional view of a syringe situated in a measurement position within a holder of the apparatus of Fig. **21**, according to one embodiment of the invention.

Fig. **22B** is a transverse sectional view of the holder and syringe of Fig. **22A**.

Fig. **23A** is a longitudinal sectional view of a syringe, holder, and capacitor arrangement, according to another embodiment of the invention.

Fig. **23B** is a transverse sectional view of the holder and syringe of Fig. **23A**.

Fig. **24A** is a perspective view of an apparatus suitable for use with a syringe, the apparatus comprising an internal capacitor, according to one embodiment of the invention.

Fig. **24B** is a longitudinal sectional view of a syringe capacitor geometry suitable for use with the apparatus of Fig. **24A**.

Fig. **25** is a high-level schematic diagram illustrating a patient monitoring and drug delivery measurement apparatus according to another embodiment of the invention.

Fig. **26** is a perspective view of a drug delivery measurement apparatus according to another embodiment of the invention.

Fig, **27A** is a longitudinal sectional view of a syringe suitable for use with the apparatus of Fig. **24A**.

Fig. **27B** is a transverse sectional view of the syringe of Fig. **27A**.

Fig. **28** is a longitudinal sectional view of an inductance-enhanced syringe of the invention.

Fig. **29A** is a longitudinal section view of a multi-inductor element according to the invention.

Fig. **29B** is a longitudinal section view of another multi-inductor element according to the invention.

Fig. **30A** illustrates qualitatively the dependence of inductance with plunger displacement for the geometry shown in Fig. **26**.

Fig. **30B** illustrates qualitatively the dependence of inductance with plunger displacement for each inductor in the geometry of Fig. **29A**.

Fig. **31A** is a high-level schematic diagram illustrating a patient monitoring and drug delivery measurement apparatus, according to another embodiment of the invention.

Fig. **31B** illustrates broadly the principal detection step performed by the apparatus of Fig. **31A**.

Fig. **32A** is a perspective view of a drug delivery measurement apparatus according to another embodiment of the invention.

Fig. **32B** is a longitudinal sectional view of a syringe situated in a measurement position in a holder of the apparatus of Fig. **32A**, illustrating a preferred light source and detector arrangement.

Fig. **32C** shows a detail of Fig. **32B**, including the plunger-liquid interface within the syringe.

Fig. **32D** shows an alternative light source and detector arrangement in a view similar to that of Fig. **32C**, according to the invention.

Fig. **32E** shows another alternative light source and detector arrangement in a view similar to that of Fig. **32C**, according to the invention.

Fig. **33A** shows an apparatus, which does not require an internal light source, according to another embodiment of the invention.

Fig. **33B** shows a longitudinal sectional view of a syringe situated in a measurement position in a holder of the apparatus of Fig. **33A**.

Fig. **34** shows a perspective view of a drug delivery measurement apparatus and a syringe adapted for use with the apparatus, according to another embodiment of the invention.

DETAILED DESCRIPTION

The invention presents a system and method for remotely monitoring individuals, for communicating information to the individuals, and for receiving data from an individual at a remote location. In a preferred embodiment of the invention, the individuals are patients and the system is used to collect data relating to the health status or medical treatment of the patients. However, it is to be understood that the invention is not limited to remote monitoring of patients or patient-related activities. The system and method of the invention may be used for any type of remote monitoring application. The invention may also be implemented as an automated messaging system for communicating information to individuals, as will be discussed in an alternative embodiment below.

A system and method for remote interactive communication and remote monitoring of individuals will first be described with reference to Figs. **1-19**. Thereafter, a system for patient monitoring and drug delivery measurement, including a drug delivery

measurement apparatus for use in conjunction with the interactive communication and remote monitoring system, will be described with reference to Figs. **20A-34**.

A preferred embodiment of the invention is illustrated in Figs. **1-12**. Referring to Fig. **1**, a networked system **16** includes a server **18** and a workstation **20** connected to server **18** through a communication network **24**. Server **18** is preferably a worldwide web server and communication network **24** is preferably the Internet. It will be apparent to one skilled in the art that server **18** may comprise a single stand-alone computer or multiple computers distributed throughout a network. Workstation **20** is preferably a personal computer, remote terminal, or web TV unit connected to server **18** via the Internet. Workstation **20** functions as a remote interface for entering in server **18** messages and queries to be communicated to the patients.

System **16** also includes a plurality of remotely programmable apparatuses, schematically represented in Fig. **1** as **26a** and **26x** for monitoring a corresponding plurality of patients. Each remotely programmable apparatus **26a-x** is designed to interact with a patient in accordance with script programs received from server **18**. Each remotely programmable apparatus **26a-x** is in communication with server **18** through communication network **24**, preferably the Internet. Alternatively, each apparatus, e.g., **26a**, may be placed in communication with server **18** via wireless communication networks, cellular networks, telephone networks, or any other network which allows each remotely programmable apparatus 26a to exchange data with server **18**. For clarity of illustration, only two remotely programmable apparatus 26aes are shown in Fig. **1**. However, it is to be understood that system **16** may include any number of remotely programmable apparatus **26a-x** for monitoring any number of patients.

Each patient to be monitored may be provided with a monitoring device **28a-x**, designed to provide measurements of a physiological condition of the patient, to record the physiological condition measurements, and to transmit the measurements to the patient's remotely programmable apparatus **26a-x**, e.g., through a standard connection cable **30**. Examples of suitable types of monitoring devices include blood glucose meters, respiratory flow meters, blood pressure cuffs, electronic weight scales, and pulse rate monitors. Such monitoring devices are well known in the art. The specific type of

monitoring device provided to each patient is dependent upon the patient's disease. For example, diabetes patients are provided with a blood glucose meters for measuring blood glucose concentrations, asthma patients are provided with respiratory flow meters for measuring peak flow rates, obesity patients are provided with weight scales, etc.

Fig. **2** shows server **18**, workstation **20**, and remotely programmable apparatus **26a-x** in greater detail. Server **18** includes a database **38** for storing script programs **40**. The script programs are executed by each remotely programmable apparatus to communicate queries and messages to a patient, receive responses **42** to the queries, collect monitoring device measurements **44**, and transmit responses **42** and measurements **44** to server **18**. Database **38** is designed to store the responses **42** and measurements **44**. Database **38** further includes a look-up table **46**. Table **46** contains a list of the patients to be monitored, and for each patient, a unique patient identification code and a respective pointer to the script program assigned to the patient. Each remotely programmable apparatus **26a-x** is designed to execute assigned script programs, which it receives from server **18**.

Figs. **3-4** show the structure of each remotely programmable apparatus according to a preferred embodiment. For clarity, only remotely programmable apparatus **26a** is shown in Figs. **3-4**, since each remotely programmable apparatus **26a-x** has substantially the same structure as remotely programmable apparatus **26a**. Referring to Fig. **3**, remotely programmable apparatus **26a** includes a housing **62**. Housing **62** is sufficiently compact to enable apparatus **26a** to be hand-held and carried by a patient. Remotely programmable apparatus **26a** also includes a display **64** for displaying queries and prompts to the patient. In a preferred embodiment, display **64** is a liquid crystal display (LCD).

Four user input buttons **70A**, **70B**, **70C**, and **70D** are located adjacent display **64**. User input buttons **70A-C** are for entering in remotely programmable apparatus **26a** responses to the queries and prompts. In a preferred embodiment, user input buttons **70A-C** are momentary contact push buttons. In alternative embodiments, user input buttons **70A-C** may be replaced by switches, keys, a touch sensitive display screen, or any other data input device.

Three monitoring device jacks **68A**, **68B**, and **68C** are located on a surface of housing **62**. The device jacks are for connecting remotely programmable apparatus **26a** to a number of monitoring devices, such as blood glucose meters, respiratory flow meters, or blood pressure cuffs, through respective connection cables (not shown). Apparatus **26a** also includes a modem jack **66** for connecting apparatus **26a** to a telephone jack through a standard connection cord (not shown). Apparatus **26a** further includes a visual indicator, such as a light emitting diode (LED) **74**. LED **74** is for visually notifying the patient that he or she has unanswered queries stored in remotely programmable apparatus **26a**.

Fig. **4** is a schematic block diagram illustrating the components of apparatus **26a** in greater detail. Apparatus **26a** includes a microprocessor **76** and a memory **80** connected to microprocessor **76**. Memory **80** is preferably a non-volatile memory, such as a serial EEPROM. Memory **80** stores script programs received from the server, measurements received from monitoring device **28**, responses to queries, and the patient's unique identification code. Microprocessor **76** also includes built-in read only memory (ROM) which stores firmware for controlling the operation of apparatus **26a**. The firmware includes a script interpreter used by microprocessor **76** to execute the script programs. The script interpreter interprets script commands, which are executed by microprocessor **76**. Specific techniques for interpreting and executing script commands in this manner are well known in the art.

Microprocessor **76** is preferably connected to memory **80** using a standard two-wire I^2C interface. Microprocessor **76** is also connected to user input buttons **70**, LED **74**, a clock **84**, and a display driver **82**. Clock **84** indicates the current date and time to microprocessor **76**. For clarity of illustration, clock **84** is shown as a separate component, but is preferably built into microprocessor **76**. Display driver **82** operates under the control of microprocessor **76** to display information on display **64**. Microprocessor **76** is preferably a PIC 16C65 processor, which includes a universal asynchronous receiver transmitter (UART) **78**. UART **78** is for communicating with a modem **86** and a device interface **90**. A CMOS switch **88** under the control of microprocessor **76** alternately connects modem **86** and interface **90** to UART **78**.

Modem **86** is connected to a telephone jack **22** through modem jack **66**. Modem **86** is for exchanging data with server **18** through communication network **24**. The data includes script programs, which are received from server **18** as well as responses to queries, device measurements, script identification codes, and the patient's unique identification code which modem **86** transmits to server **18**. Modem **86** is preferably a complete 28.8 K modem commercially available from Cermetek, although any suitable modem may be used.

Device interface **90** is connected to device jacks **68A**, **68B**, and **68C**. Device interface **90** is for interfacing with a number of monitoring devices, such as blood glucose meters, respiratory flow meters, blood pressure cuffs, weight scales, or pulse rate monitors, through device jacks **68A-C**. Device interface **90** operates under the control of microprocessor **76** to collect measurements from the monitoring devices and to output the measurements to microprocessor **76** for storage in memory **80**. In a preferred embodiment, interface **90** is a standard RS232 interface. For simplicity of illustration, only one device interface is shown in Fig. **4**. However, in alternative embodiments, remotely programmable apparatus **26a** may include multiple device interfaces to accommodate monitoring devices, which have different connection standards.

Referring again to Fig. **2**, server **18** includes a monitoring application **48**. Monitoring application **48** is a controlling software application executed by server **18** to perform the various functions described below. Application **48** includes a script generator **50**, a script assignor **52**, and a report generator **54**. Script generator **50** is designed to generate script programs **40** from script information entered through workstation **20**. The script information is entered through a script entry screen **56**. In a preferred embodiment, script entry screen **56** is implemented as a web page on server **18**. Workstation **20** includes a web browser for accessing the web page to enter the script information.

Fig. **5** illustrates script entry screen **56** as it appears on workstation **20**. Screen **56** includes a script name field **92** for specifying the name of a script program to be generated. Screen **56** also includes entry fields **94** for entering a set of queries to be answered by a patient. Each entry field **94** has corresponding response choice fields **96** for entering response choices for the query. Screen **56** further includes check boxes **98**

for selecting a desired monitoring device from which to collect measurements, such as a blood glucose meter, respiratory flow meter, or blood pressure cuff.

Screen **56** additionally includes a connection time field **100** for specifying a prescribed connection time at which each remotely programmable apparatus **26a-x** executing the script is to establish a subsequent communication link to server **18**. The connection time is preferably selected to be the time at which communication rates are the lowest, such as 3:00 AM. Screen **56** also includes a CREATE SCRIPT button **102** for instructing the script generator to generate a script program from the information entered in screen **56**. Screen **56** further includes a CANCEL button **104** for canceling the information entered in screen **56**.

In a preferred embodiment, each script program created by the script generator conforms to the standard file format used on UNIX systems. In the standard file format, each command is listed in the upper case and followed by a colon. Every line in the script program is terminated by a linefeed character {LF}, and only one command is placed on each line. The last character in the script program is a UNIX end of file character {EOF}. Table 1 shows an exemplary listing of script commands used in a preferred embodiment of the invention.

TABLE 1 - SCRIPT COMMANDS

Command	Description
CLS: {LF}	Clear the display.
ZAP: {LF}	Erase from memory the last set of query responses recorded.
LED: b{LF}	Turn the LED on or off, where b is a binary digit of 0 or 1. An argument of 1 turns on the LED, and an argument of 0 turns off the LED.
DISPLAY: {chars}{LF}	Display the text following the DISPLAY command.

INPUT: mmmm{LF}	Record a button press. The m's represent a button mask pattern for each of the four input buttons. Each m contains an "X" for disallowed buttons or an "O" for allowed buttons. For example, INPUT: OXOX{LF} allows the user to press either button #1 or #3.
WAIT: {LF}	Wait for any one button to be pressed, then continue executing the script program.
COLLECT: device{LF}	Collect measurements from the monitoring device specified in the COLLECT command. The user is preferably prompted to connect the specified monitoring device to the apparatus and press a button to continue.
NUMBER: aaaa{LF}	Assign a script identification code to the script program. The script identification code from the most recently executed NUMBER statement is subsequently transmitted to the server along with the query responses and device measurements. The script identification code identifies to the server which script program was most recently executed by the remote apparatus.
DELAY: t {LF}	Wait until time t specified in the DELAY command, usually the prescribed connection time.
CONNECT: {LF}	Perform a connection routine to establish a communication link to the server, transmit the patient identification code, query responses, device measurements, and script identification code to the server, and receive and store a new script program. When the server instructs the apparatus to disconnect, the script interpreter is restarted, allowing the new script program to execute.

The script commands illustrated in Table 1 are representative of the preferred embodiment and are not intended to limit the scope of the invention. After consideration of the ensuing description, it will be apparent to one skilled in the art many other suitable scripting languages and sets of script commands may be used to implement the invention.

Script generator **50** preferably stores a script program template, which it uses to create each script program. To generate a script program, script generator **50** inserts into the

template the script information entered in screen **56**. For example, Figs. **6A-6B** illustrate a sample script program created by script generator **50** from the script information shown in Fig. **5**.

The script program includes display commands to display the queries and response choices entered in fields **94** and **96**, respectively. The script program also includes input commands to receive responses to the queries. The script program further includes a collect command to collect device measurements from the monitoring device **28a-x** specified in check boxes **98**. The script program also includes commands to establish a subsequent communication link to server **18** at the connection time specified in field **100**. The steps included in the script program are also shown in the flow chart of Figs. **12A-12B** and will be discussed in the operation section below.

Referring again to Fig. **2**, script assignor **52** is for assigning script programs **40** to the patients. Script programs **40** are assigned in accordance with script assignment information entered through workstation **20**. The script assignment information is entered through a script assignment screen **57**, which is preferably implemented as a web page on server **18**.

Fig. **7** illustrates a sample script assignment screen **57** as it appears on workstation **20**. Screen **57** includes check boxes **106** for selecting a script program to be assigned and check boxes **108** for selecting the patients to whom the script program is to be assigned. Screen **57** also includes an ASSIGN SCRIPT button **112** for entering the assignments. When button **112** is activated, the script assignor creates and stores for each patient selected in check boxes **108** a respective pointer to the script program selected in check boxes **106**. Each pointer is stored in the patient look-up table of the database. Screen **57** further includes an ADD SCRIPT button **110** for accessing the script entry screen and a DELETE SCRIPT button **114** for deleting a script program.

Referring again to Fig. **2**, report generator **54** is designed to generate a patient report **58** from the responses and device measurements received in server **18**. Patient report **58** is displayed on workstation **20**. Fig. **10** shows a sample patient report **58** produced by report generator **54** for a selected patient. Patient report **58** includes a graph **116** of the

device measurements received from the patient, as well as a listing of responses **42** received from the patient. Specific techniques for writing a report generator program to display data in this manner are well known in the art.

The operation of a preferred embodiment is illustrated in Figs. **1-12**. Fig. **11A** is a flow chart illustrating steps included in the monitoring application executed by server **18**. Fig. **11B** is a continuation of the flow chart of Fig. **11A**. In step **202**, server **18** determines if new script information has been entered through script entry screen **56**. If new script information has not been entered, server **18** proceeds to step **206**. If new script information has been entered, server **18** proceeds to step **204**.

As shown in Fig. **5**, the script information includes a set of queries, and for each of the queries, corresponding response choices. The script information also includes a selected monitoring device type from which to collect device measurements. The script information further includes a prescribed connection time for each remotely programmable apparatus 26a to establish a subsequent communication link to server **18**. The script information is generally entered in server **18** by a healthcare provider, such as the patients' physician or case manager. Of course, any person desiring to communicate with the patients may also be granted access to server **18** to create and assign script programs. Further, it is to be understood that system **16** may include any number of remote interfaces for entering script generation and script assignment information in server **18**.

In step **204**, script generator **50** generates a script program from the information entered in screen **56**. The script program is stored in database **38**. Steps **202** and **204** are preferably repeated to generate multiple script programs, e.g. a script program for diabetes patients, a script program for asthma patients, etc. Each script program corresponds to a respective one of the sets of queries entered through script entry screen **56**. Following step **204**, server **18** proceeds to step **206**.

In step **206**, server **18** determines if new script assignment information has been entered through assignment screen **57**. If new script assignment information has not been entered, server **18** proceeds to step **210**. If new script assignment information has been

entered, server **18** proceeds to step **208**. As shown in Fig. **7**, the script programs are assigned to each patient by selecting a script program through check boxes **106**, selecting the patients to whom the selected script program is to be assigned through check boxes **108**, and pressing the ASSIGN SCRIPT button **112**. When button **112** is pressed, script assignor **52** creates for each patient selected in check boxes **108** a respective pointer to the script program selected in check boxes **106**. In step **208**, each pointer is stored in look-up table **46** of database **38**. Following step **208**, server **18** proceeds to step **210**.

In step **210**, server **18** determines if any of the remotely programmable apparatus 26aes are remotely connected to server **18**. Each patient to be monitored is preferably provided with his or her own remotely programmable apparatus **26a-x** which has the patient's unique identification code stored therein. Each patient is thus uniquely associated with a respective one of apparatus **26a-x**. If none of remotely programmable apparatus **26a-x** is connected, server **18** proceeds to step **220**.

If an apparatus **26a-x** is connected, server **18** receives from that remotely programmable apparatus the patient's unique identification code in step **212**. In step **214**, server **18** receives from the remotely programmable apparatus 26a the query responses **42**, device measurements **44**, and script identification code recorded during execution of a previously assigned script program. The script identification code identifies to server **18** which script program was executed by the apparatus **26a-x** to record the query responses and device measurements. The responses, device measurements, and script identification code are stored in database **38**.

In step **216**, server **18** uses the patient identification code to retrieve from table **46** the pointer to the script program assigned to the patient. Server **18** then retrieves the assigned script program from database **38**. In step **218**, server **18** transmits the assigned script program to the patient's remotely programmable apparatus **26a-x** through communication network **24**. Following step **218**, server **18** proceeds to step **220**.

In step **220**, server **18** determines if a patient report request has been received from workstation **20**. If no report request has been received, server **18** returns to step **202**. If a report request has been received for a selected patient, server **18** retrieves from database

38 the measurements and query responses last received from the patient, step 222. In step 224, server 18 generates and displays patient report 58 on workstation 20. As shown in Fig. 10, report 58 includes the device measurements and query responses last received from the patient. Following step 224, server 18 returns to step 202.

Figs. 12A-12B illustrate the steps included in the script program executed by remotely programmable apparatus, e.g., 26a. Before the script program is received, remotely programmable apparatus 26a is initially programmed with the patient's unique identification code and the script interpreter used by microprocessor 76 to execute the script program. The initial programming may be achieved during manufacture of apparatus 26a or during an initial connection to server 18. Following initial programming, remotely programmable apparatus 26a receives from server 18 the script program assigned to the patient associated with remotely programmable apparatus 26a. The script program is received by modem 86 through a first communication link and stored in memory 80.

In step 302, microprocessor 76 assigns a script identification code to the script program and stores the script identification code in memory 80. The script identification code is subsequently transmitted to server 18 along with the query responses and device measurements to identify to server 18 which script program was most recently executed by apparatus 26a. In step 304, microprocessor 76 lights LED 74 to notify the patient that he or she has unanswered queries stored in remotely programmable apparatus 26a. LED 74 preferably remains lit until the queries are answered by the patient. In step 306, microprocessor 76 erases from memory 80 the last set of query responses recorded.

In step 308, microprocessor 76 prompts the patient by displaying on display 64 "ANSWER QUERIES NOW? PRESS ANY BUTTON TO START". In step 310, microprocessor 76 waits until a reply to the prompt is received from the patient. When a reply is received, microprocessor 76 proceeds to step 312. In step 312, microprocessor 76 executes successive display and input commands to display the queries and response choices on display 64 and to receive responses to the queries.

Fig. 8 illustrates a sample query and its corresponding response choices as they appear on display 64. The response choices are positioned on display 64 such that each response choice is located proximate a respective one of the input buttons 70A-D. In the preferred embodiment, each response choice is displayed immediately above a respective input button 70A-D. The patient presses the button (70A-D) corresponding to his or her response. Microprocessor 76 stores each response in memory 80.

In steps 314 - 318, microprocessor 76 executes commands to collect device measurements from a selected monitoring device 28a-x. The script program specifies the selected monitoring device 28a-x from which to collect the measurements. In step 314, microprocessor 76 prompts the patient to connect a selected monitoring device 28a-x, for example a blood glucose meter, to one of device jacks 68A-C. A sample prompt is shown in Fig. 9. In step 316, microprocessor 76 waits until a reply to the prompt is received from the patient. When a reply is received, microprocessor 76 proceeds to step 318. Microprocessor 76 also connects UART 78 to interface 90 through switch 88. In step 318, microprocessor 76 collects the device measurements from monitoring device 28a-x through interface 90. The measurements are stored in memory 80.

In step 320, microprocessor 76 prompts the patient to connect remotely programmable apparatus 26a to telephone jack 22 so that remotely programmable apparatus 26a may connect to server 18 at the prescribed connection time. In step 322, microprocessor 76 waits until a reply to the prompt is received from the patient. When a reply is received, microprocessor 76 turns off LED 74 in step 324. In step 326, microprocessor 76 waits until it is time to connect to server 18. Microprocessor 76 compares the connection time specified in the script program to the current time output by clock 84. When it is time to connect, microprocessor 76 connects UART 78 to modem 86 through switch 88.

In step 328, microprocessor 76 establishes a subsequent communication link between apparatus 26a and server 18 through modem 86 and communication network 24. If the connection fails for any reason, microprocessor 76 repeats step 328 to get a successful connection. In step 330, microprocessor 76 transmits the device measurements, query responses, script identification code, and patient identification code stored in memory 80 to server 18 through the subsequent communication link. In step 332, microprocessor 76

receives through modem **86** a new script program from server **18**. The new script program is stored in memory **80** for subsequent execution by microprocessor **76**. Following step **332**, the script program ends.

One advantage of the monitoring system **16** of the present invention is that it allows each patient to select a convenient time to respond to the queries, so that the monitoring system is not intrusive to the patient's schedule. A second advantage of monitoring system **16** is that it incurs very low communications charges because each remote apparatus **26a-x** connects to server **18** at times when communication rates are lowest. Moreover, the cost to manufacture each remote apparatus **26a-x** is very low compared to personal computers or internet terminals.

A third advantage of the monitoring system is that it allows each apparatus **26a-x** to be programmed remotely through script programs. Patient surveys, connection times, display prompts, selected monitoring devices, patient customization, and other operational details of each remotely programmable apparatus **26a-x** may be easily changed by transmitting a new script program to the apparatus **26a-x**. Moreover, each script program may be easily created and assigned by remotely accessing server **18** through the Internet. Thus, the invention provides a powerful, convenient, and inexpensive system for remotely monitoring a large number of patients.

Figs. **13-15** illustrate a second embodiment of the invention in which each remotely programmable apparatus has speech recognition and speech synthesis functionality. Fig. **13** shows a perspective view of a remotely programmable apparatus **27** according to the second embodiment. Apparatus **27** includes a speaker **72** for audibly communicating queries and prompts to the patient. Apparatus **27** also includes a microphone **118** for receiving spoken responses to the queries and prompts. Apparatus **27** may optionally include a display **64** for displaying prompts to the patient, as shown in Fig. **14**.

Fig. **15** is a schematic block diagram illustrating the components of remotely programmable apparatus **27** in greater detail. Apparatus **27** is similar in design to remotely programmable apparatus **26a** of the preferred embodiment, except that remotely

programmable apparatus **27** includes an audio processor chip **120** in place of microprocessor **76**. Audio processor chip **120** is can be an RSC-164 chip, such as is commercially available from Sensory Circuits Inc., San Jose, CA.

Audio processor chip **120** has a microcontroller **122** for executing script programs received from server **18**. A memory **80** is connected to microcontroller **122**. Memory **80** stores the script programs and a script interpreter used by microcontroller **122** to execute the script programs. Memory **80** also stores measurements received from monitoring device **28**, responses to the queries, script identification codes, and the patient's unique identification code.

Audio processor chip **120** also has built in speech synthesis functionality for synthesizing queries and prompts to a patient through speaker **72**. For speech synthesis, chip **120** includes a digital to analog converter (DAC) **142** and an amplifier **144**. DAC **142** and amplifier **144** drive speaker **72** under the control of microcontroller **122**.

Audio processor chip **120** further has built in speech recognition functionality for recognizing responses spoken into microphone **118**. Audio signals received through microphone **118** are converted to electrical signals and sent to a preamp and gain control circuit **128**. Preamp and gain control circuit **128** is controlled by an automatic gain control circuit **136**, which is in turn controlled by microcontroller **122**. After being amplified by preamp **128**, the electrical signals enter chip **120** and pass through a multiplexer **130** and an analog to digital converter (ADC) **132**. The resulting digital signals pass through a digital logic circuit **134** and enter microcontroller **122** for speech recognition.

Audio processor chip **120** also includes a RAM **138** for short term memory storage and a ROM **140** which stores programs executed by microcontroller **122** to perform speech recognition and speech synthesis. Chip **120** operates at a clock speed determined by a crystal **126**. Chip **120** also includes a clock **84**, which provides the current date and time to microcontroller **122**. As in a preferred embodiment, apparatus **27** includes an LED **74**, display driver **82**, modem **86**, and device interface **90**, all of which are connected to microcontroller **122**.

The operation of the second embodiment is similar to the operation of the preferred embodiment except that queries, response choices, and prompts are audibly communicated to the patient through speaker **72** rather than being displayed to the patient on display **64**. The operation of the second embodiment also differs from the operation of the preferred embodiment in that responses to the queries and prompts are received through microphone **118** rather than through user input buttons **76A-D**.

The script programs of the second embodiment are similar to the script program shown in Figs. **6A-6B**, except that each display command is replaced by a speech synthesis command and each input command is replaced by a speech recognition command. The speech synthesis commands are executed by microcontroller **122** to synthesize the queries, response choices, and prompts through speaker **72**. The speech recognition commands are executed by microcontroller **122** to recognize responses spoken into microphone **118**.

For example, to ask the patient how he or she feels and record a response, microcontroller **122** first executes a speech synthesis command to synthesize through speaker **72** "How do you feel? Please answer with one of the following responses: very bad, bad, good, or very good." Next, microcontroller **118** executes a speech recognition command to recognize the response spoken into microphone **118**. The recognized response is stored in memory **80** and subsequently transmitted to server **18**. Other than the differences described, the operation and advantages of the second embodiment are the same as the operation and advantages of the preferred embodiment described above.

Although the first and second embodiments focus on querying individuals and collecting responses to the queries, the system of the invention is not limited to querying applications. The system may also be used simply to communicate messages to the individuals. Figs. **16-19** illustrate a third embodiment in which the system is used to perform this automated messaging function. In the third embodiment, each script program contains a set of statements to be communicated to an individual rather than a set of queries to be answered by the individual. Of course, it will be apparent to one

skilled in the art that the script programs may optionally include both queries and statements.

The third embodiment also shows how the queries and statements may be customized to each individual by merging personal data with the script programs, much like a standard mail merge application. Referring to Fig. **16**, personal data relating to each individual is preferably stored in look-up table **46** of database **38**. By way of example, the data may include each individual's name, the name of each individual's physician, test results, appointment dates, or any other desired data. As in the preferred embodiment, database **38** also stores generic script programs **40** created by script generator **50**.

Server **18** includes a data merge program **55** for merging the data stored in table **46** with generic script programs **40**. Data merge program **55** is designed to retrieve selected data from table **46** and to insert the data into statements in generic script programs **40**, thus creating custom script programs **41**. Each custom script program **41** contains statements, which are customized to an individual. For example, the statements may be customized with the individual's name, test results, etc. Examples of such customized statements are shown in Figs. **17-18**.

The operation of the third embodiment is similar to the operation of the preferred embodiment except that the script programs are used to communicate messages to the individuals rather than to query the individuals. Each message is preferably a set of statements. Referring to Fig. **19**, the statements may be entered in server **18** through script entry screen **56**, just like the queries of the preferred embodiment.

Each statement preferably includes one or more insert commands specifying data from table **46** to be inserted into the statement. The insert commands instruct data merge program **55** to retrieve the specified data from database **38** and to insert the data into the statement. For example, the insert commands shown in Fig. **19** instruct the data merge program to insert a physician name, an appointment date, a patient name, and a test result into the statements. As in the preferred embodiment, each statement may also include one or more response choices, which are entered in fields **96**.

Following entry of the statements and response choices, CREATE SCRIPT button **102** is pressed. When button **102** is pressed, script generator **50** generates a generic script program from the information entered in screen **56**. The generic script program is similar to the script program shown in Figs. **6A-6B**, except that the display commands specify statements to be displayed rather than queries. Further, the statements include insert commands specifying data to be inserted into the script program. As in the preferred embodiment, multiple script programs are preferably generated, e.g. a generic script program for diabetes patients, a generic script program for asthma patients, etc. The generic script programs are stored in database **38**.

Following generation of the generic script programs, server **18** receives script assignment information entered through script assignment screen **57**. As shown in Fig. **7**, the script programs are assigned by first selecting one of the generic script programs through check boxes **106**, selecting individuals through check boxes **108**, and pressing the ASSIGN SCRIPT button **112**. When button **112** is pressed, data merge program **55** creates a custom script program for each individual selected in check boxes **108**.

Each custom script program is preferably created by using the selected generic script program as a template. For each individual selected, data merge program **55** retrieves from database **38** the data specified in the insert commands. Next, data merge program **55** inserts the data into the appropriate statements in the generic script program to create a custom script program for the individual. Each custom script program is stored in database **38**.

As each custom script program is generated for an individual, script assignor **52** assigns the script program to the individual. This is preferably accomplished by creating a pointer to the custom script program and storing the pointer with the individual's unique identification code in table **46**. When the individual's remote apparatus **26a-x** connects to server **18**, server **18** receives from apparatus **26a-x** the individual's unique identification code. Server **18** uses the unique identification code to retrieve from table **46** the pointer to the custom script program assigned to the individual. Next, server **18** retrieves the assigned script program from database **38** and transmits the script program to the

individual's remotely programmable apparatus **26a-x** through communication network **24**.

Remotely programmable apparatus **26a-x** receives and executes the script program. The execution of the script program is similar to the execution described in the preferred embodiment, except that statements are displayed to the individual rather than queries. Figs. **17-18** illustrate two sample statements as they appear on display **64**. Each statement includes a response choice, preferably an acknowledgment such as "OK". After reading a statement, the individual presses the button corresponding to the response choice to proceed to the next statement. Alternatively, the script program may specify a period of time that each statement is to be displayed before proceeding to the next statement. The remaining operation of the third embodiment is analogous to the operation of the preferred embodiment described above.

Although it is presently preferred to generate a custom script program for each individual as soon as script assignment information is received for the individual, it is also possible to wait until the individual's apparatus connects to server **18** before generating the custom script program. This is accomplished by creating and storing a pointer to the generic script program assigned to the individual, as previously described in the preferred embodiment. When the individual's remotely programmable apparatus **26a-x** connects to server **18**, data merge program **55** creates a custom script program for the individual from the generic script program assigned to the individual. The custom script program is then sent to the individual's remotely programmable apparatus **26a-x** for execution.

Fig. **20A** is a block diagram of a networked monitoring system **16'**, according to a currently preferred embodiment of the invention. System **16'** includes server **18**, workstation **20** connected to server **18** through a communication network **24**, and at least one remotely programmable apparatus **26a-x**, essentially as described hereinabove with reference to Fig. **1**. Each programmable apparatus **26a-x** is in communication with server **18** through communication network **24**, preferably the Internet. Alternatively, each patient's programmable apparatus, e.g., **26a**, may be placed in communication with server **18** via wireless communication networks,

cellular networks, telephone networks, or any other network which allows each apparatus **26a-x** to exchange data with server **18**. It is to be understood that system **16'** may include any number of programmable apparatuses **26a-x** for monitoring any number of patients. For clarity, only two programmable apparatuses **26a-x** are shown in Fig. **20A.** Workstation **20** may take the form of a health care provider or clinician's computer **426** (Fig. **20B**).

Each remotely programmable apparatus **26a-x** and its accompanying patient monitoring and drug delivery measurement apparatus **428/428'/428''a-x** is for monitoring a patient and for recording a patient's activity. In particular, measurement apparatus **428/428'/428''** (Figs. **20B, 25, 31A,** respectively) is adapted to provide measurements of a physiological condition of the patient, to produce measurements of a patient's treatment, to record the measurements, and to transmit the measurements to the patient's programmable apparatus **26a-x,** e.g., through a standard connection cable **30**. Each programmable apparatus **26a-x** is in communication with server **18** through communication network **24**, as described hereinabove, for transmitting measurement data from measurement apparatus, e.g., **428a-x,** to workstation **20**. Examples of suitable monitoring devices include blood glucose meters, respiratory flow meters, blood pressure cuffs, electronic weight scales, and pulse rate monitors. Examples of measurements of a patient's treatment include measurements of drug dose administered or self-administered to the patient. According to a currently preferred embodiment, such measurements include dose(s) of a drug administered to the patient via a syringe.

Fig. **20B** is a high-level schematic diagram illustrating a dose administration or drug delivery measurement apparatus **428** according to the invention. Apparatus **428** records data indicative of a dose of a drug or medication delivered to a patient from a syringe (e.g., syringe **580,** Fig. **21**). Apparatus **428** is capable of downloading the recorded data to a remotely programmable apparatus **26a-x** (Fig. **20A**).

According to one embodiment of the invention, remote apparatus **26a-x** may take the form of a patient's computer **424**, which in turn is capable of communicating with a clinician's computer **426** over a long-distance communication line such as a telephone line or the Internet,

as shown in Fig. **20B**. However, it is to be understood that apparatus **428** may be connected to other types of remote apparatus **26a-x**, and thence via a communications network to a remote interface, as described hereinabove with reference to Figs. **1-19** and elsewhere herein.

Apparatus **428** includes a dose measurement element, in the form of a capacitive element **422** enclosing at least part of a syringe (e.g., syringe **580**, Fig. **21**). According to other embodiments of the invention, a dose measurement element may take other, distinct forms. For example, a dose measurement element may be in the form of an inductive element (Figs. **25, 29A, 29B**). Capacitive element **422** includes one or more capacitors arranged in a predetermined spatial relationship. A measuring device **432** is in electrical communication with capacitive element **422**, and detects a capacitive response of capacitive element **422** when the syringe is in a predetermined measurement position. Measuring device **432** preferably includes a LC circuit with a resonant frequency $\omega = 1/\sqrt{LC}$. Capacitance-measuring devices are well known in the art. The capacitive response of capacitive element **422** is indicative of the quantity of liquid in the syringe, and consequently of the dose administered to the patient using the syringe. A control device **434** is in electrical communication with measuring device **432**, and temporally controls the operation of measuring device **432**. Control device **434** is capable of turning-on measuring device **432** when a syringe is in the measurement position, for example before the administration of the dose to the patient. Control device **434** preferably includes a button, which the patient can press to trigger a measurement.

A computing device **436** is in electrical communication with measuring device **432** and with a calibration memory **438**. Computing device **436** preferably includes a

microprocessor. Computing device **436** is further in electrical communication with a recording device **440**. Recording device **440** preferably includes a memory chip. Computing device **436** generates dose data to be stored in recording device **440**. The dose data preferably includes a dose (e.g. an insulin dose) administered to the patient, but may be in general any data which can be used to reconstruct (for example, within system **428**, at patient computer **24**, or at clinician computer **426**) the dose administered to the patient. In particular, computing device **436** calculates the quantity of liquid **592** within the syringe before injection of a dose, or the difference between the liquid quantities within the syringe before and after injection. Computing device **436** then sends the result (the dose) to recording device **440** for storage.

According to the embodiment of Fig. **20B**, computing device **436** determines liquid quantities by comparing capacitive response data received from measuring device **432** with predetermined calibration data stored in calibration memory **438**. The calibration data is indicative of the correspondence between capacitive responses and liquid quantities for the entire range of potential liquid quantities in the syringe. That is, calibration memory **438** stores the quantity of liquid **592** corresponding to a given capacitive response of capacitive element **422**, for all liquid quantities potentially present in the syringe.

A monitoring device **444** is electrically connected to recording device **440**. Monitoring device **444** tests a physical or physiological condition of the patient, and generates condition data representative of the physical or physiological condition. Preferably, the condition is diabetes, the monitoring device includes a blood glucose meter, and the condition data includes a blood glucose level of the patient. Recording device **440** records the condition data generated by monitoring device **444**. A display **546** is electrically connected to recording device **440**, and displays dose data and condition data to the patient. Display **446** can be a liquid crystal display (LCD). A display such as display **446** may be directly connected to computing device **436** and monitoring device **444**, rather than indirectly through recording device **440**. A digital clock **448** is

connected to recording device **440**. Upon prompting, clock **448** sends the current date and time to recording device **440** for recording in conjunction with dose and/or condition data.

Fig. **21** shows a perspective view of a measurement apparatus **528**, according to a preferred embodiment of the present invention. Apparatus **528** includes a housing **550** enclosing the various electronic components of apparatus **528**. Housing **550** preferably includes a metal layer for shielding internal components of apparatus **528** from external electric fields, and in particular the capacitive components of apparatus **528** (see below). As is apparent to the skilled artisan, care should also be taken to minimize all stray capacitances. Housing **550** is sufficiently compact to allow apparatus **528** to be hand-held and carried by a user.

Display **446** is preferably recessed within housing **550**. A patient interface **558** (of monitoring device **444**, Fig. **20B**) is also coupled to housing **550**. In a preferred embodiment, the patient places a finger on patient interface **558**, allowing monitoring device **444** to perform a blood glucose measurement for the patient. Blood glucose meters are well known in the art and will not be discussed here in detail. A dose measurement control **560** (of control means **434**, Fig. **20B**) is coupled to housing **550**, and allows the patient to specify when dose measurements are to be performed by apparatus **528** (see below). A port **562** allows data transfer between recording device **440** and patient computer **424** (Fig. **20B**).

Housing **550** also encloses a holder **552** for receiving and snugly holding a syringe **580** in the measurement position. A circular opening **553** within housing **550** provides access to holder **552**. Holder **552** has a well-like shape for laterally enclosing syringe **580**. Holder **552** defines an enclosed space **556** opposite opening **553**, for accommodating a needle **582** of syringe **580** when syringe **580** is in the measurement position. Syringe **580** can be a conventional syringe, such as a plastic syringe. Syringe **580** includes a barrel **586** and a plunger **590**, defining a space for a liquid **592**. Liquid **592** preferably

includes insulin. Plunger **590** is capable of longitudinal motion relative to barrel **586**, for adjusting the volume available to liquid **592**. Holder **552** includes an alignment ledge **554** for aligning barrel **586** to holder **552** in the measurement position. A contact surface **584** of syringe **580** is in contact with alignment ledge **554** when syringe **580** is in the measurement position.

Fig. **22A** shows a longitudinal sectional view through syringe **580** and holder **552**, with syringe **580** in the measurement position. Fig. **22B** shows a transverse sectional view of the arrangement of Fig. **22A**. A capacitive element **600** consists of a single capacitor defined between an electrode **600a** and liquid **592**. Electrode **600a** is a cylindrical copper sheet embedded in a plastic side wall of holder **552**, and is electrically connected to measuring device **432** (Fig. **20B**). Electrode **600a** encloses syringe **580** externally and laterally. Liquid **592** is connected to measuring device **432** through needle **582** and a needle contact **583** coupled to holder **552**. Needle contact **583** is a corrosion-resistant metal block having a sloped (conical) side wall **585** for contacting needle **582** when syringe **580** is in the measurement position.

The dielectric constant within capacitive element **600** is relatively spatially invariant and does not change substantially with the quantity of liquid **592**. The dielectric constant within capacitive element **600** is determined by the materials and/or thicknesses of barrel **586**, the air between barrel **586** and the side wall of holder **552**, and the portion of the side wall of holder **552** between electrode **600a** and barrel **586**. Neglecting edge effects and effects stemming from the non-ideal conductivity of liquid **592**, the capacitance of capacitive element **600** is then primarily determined by its surface area, which is proportional to the longitudinal extent x of liquid enclosed by electrode **600a**.

To operate measurement apparatus **528**, a patient inserts the manufacturer-provided syringe **580** in holder **552** prior to administration of the dose. When syringe **580** is pressed against alignment ledge **554** and needle **582** contacts needle contact **583**, syringe **580** is in the measurement position. The patient presses dose measurement

control (e.g., button) **560** (Fig. **21**) to activate measuring device **432**. Measuring device **432** performs a measurement of the capacitance of capacitive element **600**. Computing device **436** then determines the quantity of liquid **592** within syringe **580**. Recording device **440** records the liquid quantity as the administered dose, in conjunction with the current date and time obtained from clock **448**. Recording device **440** may also record condition data received from monitoring device **444**, and the associated date and time. Recording device **440** then contains the patient's blood glucose and insulin dose histories. The patient periodically (e.g. weekly) connects his or her measurement apparatus **528** to patient computer **424** and downloads the histories stored in recording device **440**. The histories may then be periodically transmitted to clinician's computer **426**.

Fig. **23A** shows a longitudinal sectional view of an alternative capacitive element geometry of the present invention, while Fig. **23B** shows a transverse sectional view of the geometry of Fig. **23A**. A capacitive element **700** is coupled to holder **552**, and is situated completely externally to syringe **580**. Capacitive element **700** includes a plurality of independent, longitudinally spaced, stacked capacitors **700a-e**. Each capacitor **700a-e** is independently connected to measuring device **432** (Fig. **20B**), and measuring device **432** determines the capacitive response of each capacitor **700a-e** independently. A capacitive response pattern of capacitive element **700** (the ensemble of capacitive responses of capacitors **700a-e**) is indicative of the quantity of liquid **592** within syringe **580**. The use of plural stacked capacitors reduces the vulnerability of a system of the present invention to dosage determination errors caused by a constant capacitance offset.

Capacitor **700a** includes electrodes **700a', 700a"** embedded within the side wall of holder **552** on opposite sides of syringe **580**. Capacitors **700b-e** are similar to capacitor **700a** and are stacked above capacitor **700a**. The surface area of each capacitor **700a-e** is constant, and does not depend on the quantity of liquid **592**. The effective dielectric constant of each capacitor **700a-e** may depend, however, on the quantity of liquid **592**. If liquid **592** is substantially conductive, it behaves like an

electrode inserted between electrodes **700a'** and **700a"**, thus creating two capacitors in series: one defined by electrode **700a'** and liquid **592**, the other defined by liquid **592** and electrode **700a"**. Preferably, the dielectric properties of barrel **586**, plunger **590** and liquid **592** are such that the capacitance response pattern of capacitive element **700** is indicative of the position of plunger **590** relative to capacitive element **700** and/or of the quantity of liquid **592** within syringe **580**.

Fig. **24A** shows a perspective view of an alternative apparatus **820** of the present invention, suitable for measuring doses delivered by syringes using either capacitors or inductors for dose measurement (Figs. **22A-23B** and **27A-28**, respectively). A circular placement field **870** is delineated on the outside of a housing **850** of apparatus **820**. Placement field **870** is bordered on four sides by a set of rigid positioning studs **872** forming a holder. Placement field **870** includes a circular input contact **874** positioned at the center of field **870**, and a ring-shaped output contact **876** positioned concentrically to input contact **874**. Input contact **874** and output contact **876** are made of an electrically conductive material, preferably copper, and are connected to measuring device **432** (Fig. **20B**) or measuring device **432'** (Fig. **25**).

Fig. **24B** shows a longitudinal sectional view of a syringe **880** suitable for use with the apparatus of Fig. **24A**. Syringe **880** includes a plunger **890** positioned within a barrel **886**. Plunger **890** includes a cylindrical cap **878** sized so as to fit on placement field **870** between studs **872** when syringe **880** is in a measurement position. Cap **878** includes an input terminal **812** and an output terminal **814** situated such that input terminal **812** and output terminal **814** are in electrical communication respectively with input contact **874** and output contact **876** when syringe **880** is in the measurement position.

A metallic contact line **801** within plunger **890** establishes electrical communication between output terminal **814** and a cylindrical electrode **800a'** situated embedded within the plastic body of plunger **890**, along the outside surface of plunger **890**. A second

cylindrical electrode **800a"** is encapsulated in the plastic body of barrel **886**, and is co-axial with electrode **800a'**. Electrode **800a"** is in electrical communication with input terminal **812** through metallic contact lines **802** and **804**. Line **802** is situated on the lateral (outside) surface of plunger **890**, while line **804** is situated within barrel **886**. A sliding electrical contact schematically illustrated as **803** is established between a fixed exposed point of line **804** and various points of line **802** as plunger **890** is moved within barrel **886**.

The following discussion is intended to illustrate a measurement apparatus of the invention, which utilizes a capacitive element, and should not be construed to limit the invention. Consider the geometry of Figs. **22A** and **22B**, for a typical syringe. Neglecting edge effects, the capacitance of capacitive element **600** is approximately

$$C \approx 2\pi\varepsilon_{bar} x \ln(\frac{b}{a}) \qquad [1]$$

where **x** is the length of capacitor **600**, **a** and **b** are the radii of the cylinders defined respectively by liquid **592** and electrode **600a**, and e_{bar} is the effective dielectric constant between liquid **592** and electrode **600a**. From eq. [1] it can be seen that **dC/dx**, the variation of the capacitance of capacitor **600** with displacement **x**, can be maximized for given radii **a** and **b** by increasing e_{bar}. Thus, materials with high dielectric constants are preferred for the space between liquid **592** and electrode **600a**.

Fig. **25** is a high-level schematic diagram illustrating a dose administration or drug delivery measurement apparatus **428'** according to another embodiment of the instant invention. As for apparatus **428** described hereinabove with reference to Figure **20B**, apparatus **428** records data indicative of dose(s) delivered to a patient from a syringe (e.g., syringe **580'**). Apparatus **428'** is capable of downloading the recorded data to a remotely programmable apparatus (e.g., apparatus **26a-26x**, (Figs. **1, 20A**). According to the invention, a remotely programmable apparatus **26a-26x** may take the form of a

patient computer **424** (Fig. **20B**), which in turn is capable of communicating with a clinician's computer **426** (Fig. **20B**) over a communication network or long-distance communication line, essentially as described hereinabove with reference to Figs. **1-20B**.

Apparatus **428'** includes an inductive element **423** enclosing at least part of the syringe. Inductive element **423** includes one or more inductors arranged in a predetermined spatial relationship. A measuring device **432'** is in electrical communication with inductive element **423**, and detects an inductive response of inductive element **423** when the syringe is in a predetermined measurement position. Measuring device **432'** preferably includes a LC circuit with a resonant frequency $\omega = 1/\sqrt{LC}$. Inductance-measuring devices are well known in the art. The inductive response of inductive element **423** is indicative of the quantity of liquid in the syringe, and consequently of the dose administered to the patient using the syringe. Apparatus **428'** further includes a control device **434'** in electrical communication with a measuring device **432'**; a computing device **436'** in electrical communication with measuring device **432'** and with a calibration memory **438'**; and a recording device **440'**, somewhat analogous to apparatus **428**, described hereinabove with reference to Fig. **20B**. Computing device **436'** is further in electrical communication with a recording device **440'**.

Computing device **436'** preferably includes a microprocessor. Recording device **440'** preferably includes a memory chip. Computing device **436'** generates dose data to be stored in recording device **440'**. The dose data preferably includes a dose (e.g. an insulin dose) administered to the patient, essentially as described hereinabove with reference to apparatus **428**. Computing device **436'** sends the result (the dose) to recording device **440'** for storage.

Computing device **436'** determines liquid quantities by comparing inductive response data received from measuring device **432'** with predetermined calibration data stored in calibration memory **438'**. The calibration data is indicative of the correspondence

between inductive responses and liquid quantities for the entire range of potential liquid quantities in the syringe. That is, calibration memory **438'** stores the liquid quantity corresponding to a given inductive response of inductive element **423**, for all liquid quantities potentially present in the syringe.

Apparatus **428'** further includes a monitoring device **444'** electrically connected to recording device **440'**, a display **446'** also electrically connected to recording device **440'**, and a digital clock **448'** connected to recording device **440'**, analogous to the corresponding components of apparatus **428** described hereinabove with reference to Fig. **20B**. Upon prompting, clock **448'** sends the current date and time to recording device **440'** for recording in conjunction with dose or condition data related to the patient being monitored.

Fig. **26** shows, in perspective view, an apparatus **528'**, according to one embodiment of the present invention. Apparatus **528'** includes a housing **550'** enclosing the various electronic components of apparatus **528'**. Housing **550'** preferably includes a metal layer for magnetically shielding internal components of apparatus **528'**, in particular inductive components of apparatus **528'** (see below). Housing **550'** is compact and has dimensions and weight characteristics similar to those described hereinabove with respect to housing **550** of Fig. **21**.

Housing **550'** includes a (recessed) display **446'** and a patient interface **558'** (of monitoring device **444'**, Fig. **25**) coupled to housing **550'**, similar to housing **550** of Fig. **21**. In a preferred embodiment, the patient places a finger on patient interface **558'**, allowing monitoring device **444'** to perform a blood glucose measurement for the patient. A dose measurement control **560'** (of control device **434'**, Fig. **25**) is coupled to housing **550'**, and allows the patient to specify when dose measurements are to be performed by apparatus **528'** (see below). A port **562'** allows data transfer between recording device **440'** and a patient computer or remotely programmable apparatus (not shown).

Housing **550'** also encloses a holder **552'** for receiving and holding a syringe **580'** in the measurement position. Syringe **580'** corresponds specifically to the calibration data stored in calibration memory **438'**. A circular opening **553'** within housing **550'** provides access to holder **552'**. Holder **552'** has a well-like shape for laterally enclosing syringe **580'**. Holder **552'** defines an enclosed space **556'** opposite opening **553'**, for accommodating a needle **582'** of syringe **580'** when syringe **580'** is in the measurement position. Enclosed space **556'** is preferably closed off, so as to prevent accidental access to needle **582'** while syringe **580'** is in the measurement position.

Syringe **580'** includes a barrel **586'** and a plunger **590'**, defining a space for a liquid **592**. Plunger **590'** preferably includes a longitudinal strip **591'** made of a ferromagnetic material such as ferrite, embedded in a plastic body **593'**. Alternatively, a standard syringe with a plunger consisting of a plastic body can be used with a device of the present invention. Plunger **590'** is capable of longitudinal motion relative to barrel **586'**, for adjusting the volume available to liquid **592**. Holding device **552'** includes an alignment ledge **554'** for aligning barrel **586'** to holder **552'** in the measurement position. A contact surface **584'** of syringe **580'** is in contact with alignment ledge **554'** when syringe **580'** is in the measurement position.

A cylindrical inductor **564** is mechanically coupled to holder **552'**, and encloses syringe **580'** externally and laterally. Inductor **564** encloses syringe **580'** along the entire longitudinal extent along which the position of plunger **590'** and/or the quantity of liquid **592** may vary. The position of plunger **590'** and/or the quantity of liquid **592** within syringe **580'** determine the inductance of inductor **564**. The magnetic permeabilities of plunger **590'** and liquid **592** are substantially different, such that the inductance of inductor **564** is indicative of the position of plunger **590'** relative to inductor **564** and of the quantity of liquid **592** within syringe **580'**. In particular, the two permeabilities differ by at least a factor of two, preferably by a factor of ten or more.

The operation of apparatus **528'** by a patient is similar to the operation of apparatus **528** as described hereinabove. Briefly, a patient inserts the manufacturer-provided syringe **580'** in holder **552'** prior to administration of the dose. When syringe **580'** is pressed against alignment ledge **554'**, syringe **580'** is in the measurement position. The patient presses dose measurement control (button) **560'** to activate measuring device **432'**. Measuring device **432'** (Fig. **25**) performs measurement of the inductance of inductor **564**. Computing device **436'** then determines the quantity of liquid **592** within syringe **580'**. Recording device **440'** records liquid quantity as the administered dose, in conjunction with current date and time from clock **448'**. Recording device **440'** may also record condition data received from monitoring means **444'**, and the associated date and time. The patient periodically connects apparatus **528'** to a remotely programmable apparatus **26a-x** or to a patient computer (not shown) and downloads the patient's blood glucose and insulin dose histories stored in recording device **440'** for periodic transmission to, e.g., a clinician's computer (also not shown).

Fig. **27A** shows a longitudinal sectional view of a syringe **980,** according to one embodiment of the invention, while Fig. **27B** shows a transverse sectional view of syringe **980**. Syringe **980** is suitable for use with the apparatus of Fig. **24A**, and includes a plunger **990** positioned within a barrel **986**. Plunger **990** includes a cylindrical ferromagnetic core **991** encapsulated within a plastic shell **993**. Plunger **990** also includes a cylindrical cap **978** sized so as to fit on placement field **470** (Fig. **24A**) between studs **472** when syringe **980** is in a measurement position. Cap **978** includes an input terminal **912** and an output terminal **914** situated such that input terminal **912** and output terminal **914** are in electrical communication respectively with input contact **474** and output contact **476** when syringe **980** is in the measurement position. Barrel **986** includes an inductor **900** encapsulated within a plastic side wall. Metallic contact lines **903, 905** (located within plunger **990** and barrel **986**) establish electrical communication between inductor **900** and input terminal **912** and output terminal **914**, respectively. For taking a measurement, the patient places syringe **980** on placement field **470** and activates measuring device **432'** to measure the inductance of inductor **900**.

Fig. **28** shows a longitudinal sectional view of an alternative geometry for an inductance-enhanced syringe **1080** of the present invention. A plunger **1090** of syringe **1080** includes an inductance-enhancing element **1091** having a plurality of longitudinally spaced distinct segments **1095**, embedded in a plastic shell **1093**. Segments **1095** are thin ferromagnetic disks stacked along the longitudinal direction of plunger **1090**. Segments **1095** have a magnetic permeability substantially higher than that of plastic shell **1093** or liquid **592**.

Figs. **29A** and **29B** illustrate an alternative inductor geometry of the present invention. An inductive element **1100**, coupled to and enclosing holder **552'**, is formed by a plurality of independent longitudinally-spaced, stacked inductors **1101a-c**. Measuring device **432'** is used to measure an inductive response pattern of inductors **1101a-c**. The response pattern preferably consists of the inductance of each inductor **1101a-c**. The longitudinal extent of each inductor **1101a-c** is much greater than the desired resolution achievable with inductive element **1100**. In the embodiment illustrated in Fig. **29B**, the longitudinal extent of each inductor **1201a-p** of an inductive element **1200** is of the same order of magnitude as the desired resolution to be achieved with inductive element **1200**. The use of plural longitudinally stacked inductors **1201a-p** reduces the vulnerability of a system of the present invention to dosage determination errors caused by a constant inductance offset.

Figs. **30A** and **30B** illustrate qualitatively the variation of inductance with plunger displacement for the inductive element geometries of Figs. **26** and **29A**, respectively. In Figs. **30A** and **30B**, the plunger is taken to have a higher effective magnetic susceptibility than the liquid in the syringe. The inductance of inductor **564** increases with the extent of plunger **590'** enclosed by inductor **564**, as illustrated in Fig. **30A**. The plunger displacement and delivered dose are computed from the measured inductance and stored calibration data. Likewise, the response pattern of inductive element **1100** changes as more of plunger **590'** becomes enclosed by inductive element **1100**. For a small dose to

be delivered (small plunger displacement), plunger **590'** is enclosed only by inductor **1101a** but not by inductors **1101b-c**. For a large dose to be delivered (large plunger displacement, or plunger pushed in close to the syringe needle), plunger **590'** is completely enclosed by inductors **1101a-b** and only partially enclosed by inductor **1101c**.

The following discussion is intended to illustrate a measurement apparatus of the invention, which utilizes an inductive element, and should not be construed to limit the invention. Consider a geometry similar to those shown in Figs. **26** or **27A**, for a typical inductance-enhanced syringe. Consider a position of plunger **990** in which an inductor encloses a length **x** of liquid and a length **D-x** of plunger (see Fig. **27A**). From the inductance of an ideal long solenoid of length **D**, cross-sectional area **A**, and turn density (number of turns per unit length) **n**,

$$L = \mu n^2 DA, \qquad [2]$$

one can obtain an order-of-magnitude estimate of the total inductance of the inductor as a function of **x**. Neglecting edge effects,

$$L_{tot} \approx L_{liq} + L_{plg} \approx n^2 A(\mu_{liq} x + \mu_{plg}(D - x)) = n^2 A(x(\mu_{liq} - \mu_{plg}) + \mu_{plg} D), \qquad [3]$$

where μ_{liq} and μ_{plg} are magnetic permeabilities, and L_{liq} and L_{plg} are inductances corresponding respectively to the liquid and the plunger. As illustrated by eq. [3], the sensitivity of L_{tot} to variations in **x** increases with the difference between the magnetic permeabilities of the plunger and the liquid.

For a ferromagnetic-core plunger and corresponding solenoid, **n** is on the order of 10^5 m^{-1}, **A** is on the order of 10^{-5} m^2, and μ_{liq} and μ_{plg} are, respectively, on the order of 10 μ_o for water and $>10^3$ μ_o for a typical ferromagnetic or ferrimagnetic material. The above

values yield a sensitivity on the order of 10^2 mH per mm of plunger displacement. The inductor sensitivity may be lower for a ferrite-strip plunger or for a conventional plastic plunger. The inductor sensitivity may also be lower for other inductor geometries. The corresponding sensitivity of the inductance-measuring device may be adjusted according to the particular inductor geometry and plunger used; generally a more sensitive measuring device will be needed for measuring displacements of plungers having an effective permeability closer to that of the liquid.

Fig. **31A** is a high-level schematic diagram illustrating a measurement apparatus **428''** according to another embodiment of the invention. Optical connections are indicated in Fig. **31A** by dashed lines, while electrical connections are indicated by solid lines. Apparatus **428''** records data indicative of doses delivered to a patient using a syringe **580''**. Apparatus **428''** is capable of downloading the recorded data to a remotely programmable apparatus **26a-x** (Figs. **1, 20A**), such as a patient computer **424** (Fig. **20B**), which in turn is capable of communicating with workstation **20** (Figs. **1, 20A**), or a clinician's computer **426** (Fig. **20B**) over a communication network, telephone line, or the Internet.

Apparatus **428''** includes a light source **430** and an optical detector **450** for optical communication with syringe **580''** when the latter is in a measurement position. Light source **430** generates light incident on syringe **580''**. Optical detector **450** detects an optical response of syringe **580''** to the light generated by light source **430**. The optical response of syringe **580''** is indicative of the quantity of liquid in syringe **580''**, and consequently of the dose administered to the patient using syringe **580''**. A control means **434''**, in electrical communication with light source **430** and optical detector **450**, temporally controls the operation of light source **430** and optical detector **450**. Control means **434''** turns on light source **430** and optical detector **450** when syringe **580''** is appropriately positioned for dose measurements, before and after the administration of the dose to the patient.

A computing means **436''** is in electrical communication with optical detector **450** and with a calibration memory **438''**. Computing means **436''** is further in electrical communication with a recording means **440''**. Computing means **436''** generates dose data to be stored in recording means **440''**. The dose data preferably includes a dose (e.g. an insulin dose) administered to the patient, but may be in general any data which can be used to reconstruct (for example within apparatus **428''**, at patient computer **424**, or at clinician computer **426**) the dose administered to the patient. In particular, computing means **436''** calculates quantities of liquid within syringe **580''** before and after injection of a dose. Computing means **436''** then calculates the difference between the two measured liquid quantities, and sends the result (the dose) to recording means **440''** for storage. Computing means **436''** determines liquid quantities by comparing optical response data received from optical detector **450** with predetermined calibration data stored in calibration memory **438''**. The calibration data is indicative of the correspondence between optical responses and liquid quantities for the entire range of potential liquid quantities in syringe **580''**. That is, calibration memory **438''** stores the liquid quantity corresponding to a given optical response of detector **450**, for all liquid quantities potentially present in syringe **580''**.

A monitoring or testing means **444''** is electrically connected to recording means **440''**. Monitoring means **444''** tests a physical or physiological condition of the patient, and generates condition data representative of the condition. The condition may be, for example, diabetes; the monitoring means may include a blood glucose meter; and the condition data may include a blood glucose level of the patient. Recording means **440''** records the condition data generated by monitoring means **444''**. A display **446''** is electrically connected to recording means **440''**, and displays dose data and condition data to the patient. Note that a display such as display **446''** may be directly connected to computing means **436''** and monitoring means **444''**, rather than indirectly through recording means **440''**.

Fig. **31B** illustrates generally the principal detection step performed by measurement apparatus **428''** of the present invention. Light (electromagnetic radiation) is incident on syringe **580''** and interacts with syringe **580''**. Light resulting from the interaction is then incident on detector **450** (Fig. **31A**). The light incident on detector **450** may generally be light transmitted, reflected, and/or emitted by syringe **580''**. In general, two elements of syringe **580''** may vary with the quantity of liquid within syringe **580''** in a typical dose administration sequence: i) the position of the syringe plunger (relative to the syringe barrel), and ii) the quantity/position of the liquid within syringe **580''**. Light incident on syringe **580''** may interact with the plunger and/or liquid. The measured light interaction with the plunger is preferably substantially different from the interaction with the liquid, such that the interaction with syringe **580''** as a whole depends on at least one of the position of the plunger and the quantity of liquid.

Fig. **32A** shows a perspective view of an apparatus **528''**, according to a preferred embodiment of the present invention. Apparatus **528''** includes a housing **550''** enclosing the various electronic and optical components of apparatus **528''**. Display **446''** is recessed within housing **550''**. A patient interface **558''** (of monitoring means **444''**, Fig. **31A**) is also coupled to housing **550''**. In a preferred embodiment, the patient places his or her finger on patient interface **558''**, allowing monitoring means **444''** to perform a blood glucose measurement for the patient. A dose measurement control **560''** of control means **434''** is coupled to housing **550''**, and allows the patient to specify when dose measurements are to be performed by apparatus **528''** (see below).

Housing **550''** also encloses a holding means **552''** for receiving and holding a syringe **580''** in a measurement position. Syringe **580''** can be a conventional plastic syringe. Syringe **580''** includes a barrel **586''** and a plunger **590''**, defining a space for a liquid **592**. Plunger **590''** is capable of longitudinal motion relative to barrel **586''**, for adjusting the volume available to liquid **592**. Barrel **586''** has side walls transparent at a

wavelength of light emitted by light source **430** (Fig. **31A**), as well as a control portion **588''** opaque at a wavelength of light emitted by a control emitter (see below).

Holding means **552''** includes an alignment ledge **554''** for aligning barrel **586''** to holding means **552''** in a predetermined measurement position. A contact surface **584''** of syringe **580''** is in contact with alignment ledge **554''** when syringe **580''** is in the measurement position (see below). A space **556''** accommodates a needle **582''** of syringe **580''**, when the latter is in the measurement position.

Fig. **32B** shows a longitudinal sectional view through syringe **580''** and holding means **552''**, with syringe **580''** in a measurement position. A light source **1300** and an optical detector **1302** are mechanically coupled to holding means **552''**, and in optical communication with syringe **580''**. Optical detector **1302** is opposite light source **1300** relative to syringe **580''**, such that optical detector **1302** detects light transmitted through syringe **580''**. Light source **1300** generates light incident on both plunger **590''** and liquid **592**. A control light source **1304** and a control optical detector **1306** of control means **434''** (Fig. **31A**) are mechanically coupled to holding means **552''**, and are in optical communication with control portion **588''** (Fig. **32A**) when syringe **580''** is in the measurement position.

Fig. **32C** shows a detail of Fig. **32B**. Following a patient command entered by the patient pressing dose measurement control **560''** (Fig. **32A**), control light source **1304** emits a light beam **1309** which is blocked by control portion **588''** when syringe **580''** is in the measurement position. If light beam **1309** is blocked, control means **434''** operates light source **1300** and detector **1302** to take a first liquid quantity measurement, before the injection of liquid **592** by the patient. Light beam **1309** is then incident on control detector **1306** while syringe **580''** is out of holding means **552''**. When the patient inserts syringe **580''** into holding means **552''** after the injection of a dose of liquid **592**, light beam **1309** is again blocked, and control means **434''** operates light source **1300** and detector **1302** to take a second liquid quantity measurement. The difference between the

two liquid quantities is taken to be the dose injected by the patient, and is stored by recording means **440''**.

Light source **1300** includes a plurality of light emitters **1300a-f**, while detector **1302** includes a plurality of detecting elements **1302a-f**. Light emitters **1300a-f** and detecting elements **1302a-f** are longitudinally spaced apart at regular intervals. Each light emitter **1300a-f** is longitudinally aligned to a corresponding detecting element **1302a-f**. Light emitters **1300a-f** are preferably narrow-angle light emitting diodes (LEDs), while detecting elements **1302a-f** are preferably photodiodes capable of detecting light of a wavelength emitted by light emitters **1300a-f**.

For detecting the quantity of liquid **592** within syringe **580''**, light emitters **1300a-f** emit light beams **1308a-f** incident on plunger **590''** and liquid **592**. Detector elements **1302a-f** detect the resulting optical response pattern of syringe **580''**. Emitter **1300d**, situated under the current position of plunger **590''**, emits a light beam **1308d** which passes through liquid **592** and is incident on detector **1302d**. Emitter **1300e**, situated above the current position of plunger **590''**, emits a light beam **1308e** which is incident on plunger **590''**. Plunger **590''** has a substantially different optical transmission property from liquid **592** at the wavelength(s) measured by detecting element **1302e**. Preferably, plunger **590''** is opaque at those wavelengths. Plunger **590''** then substantially blocks beam **1308e**, such that beam **1308e** is not incident on detecting element **1302e**. An electrical signal indicative of the optical pattern detected by detector **1302** is sent to computing means **436''**.

Fig. **32D** illustrates an alternative geometry for a detector of the present invention. A detector **1352** includes detecting elements **202a-c**, each of which receives light emitted by plural emitters of light source **1300**. Fig. **32E** illustrates yet another geometry for a light source and detector of the present invention. A light source **1300'** and a detector **1352'** each comprise a single emitting or detecting element, extending longitudinally over the range of potential plunger bottom positions. The total amount of

light detected by detector **1352'** is indicative of the plunger position – relatively little light is incident on detector **1352'** if the plunger occludes the light path between light source **1300'** and detector **1352'**. The single-element detecting scheme illustrated in Fig. **32E** can be less sensitive than a multiple-element detecting scheme using similar components, but is advantageous because of its simple design.

Fig. **33A** shows a perspective view of another embodiment of the present invention. An apparatus **1420** includes a holding means **1452** which encloses syringe **580''** only on one side when syringe **580''** is in a measurement position. Fig. **33B** shows a longitudinal side view of the holding means **1452** and syringe **580''** in the measurement position. A control ledge **1454** aligns barrel **586''** of syringe **580''** with a detector **1402** in the measurement position. Detector **1402** includes plural longitudinally spaced detecting elements **1402a-x**. To take measurements, the patient orients the measurement face of holding means **1452** toward an external source of spatially uniform light, preferably a parallel light beam. For example, the patient places apparatus **1452** close to a bright window or lamp. For the embodiment in Fig. **33A**, the computing means calculates quantities of liquid within syringe **580''** according to the distribution of signals received from the detecting elements of detector **1402**, rather than the absolute values of the signals.

Fig. **34** shows a perspective view of another embodiment of the present invention. An apparatus **1528** includes a holding means **1552** for holding the barrel of a syringe **1580** in a predetermined position relative to a measurement window **1503**. Syringe **1580** includes a plunger **1590** having a longitudinally varying marking **1591**. Marking **1591** is desirably a color marking, but generally may be a shape marking. A light source and detector (both not shown) are situated behind measurement window **1503**, for reading the part of marking **1591** in alignment with window **1503**. Light emitted by the light source is reflected by marking **1591** back into the detector. The reflected light (its intensity and/or spatial distribution) is indicative of the position of marking **1591** relative to window **1503**, which is in turn indicative of the quantity of liquid within syringe **1580**.

The quantity of liquid within syringe **1580** is in turn indicative of a dose delivered from syringe **1580**.

SUMMARY, RAMIFICATIONS, AND SCOPE

Although the above description contains many specificities, these should not be construed as limitations on the scope of the invention but merely as illustrations of some of the presently preferred embodiments. Many other embodiments of the invention are possible. For example, the scripting language and script commands shown are representative of the preferred embodiment. It will be apparent to one skilled in the art that many other scripting languages and specific script commands may be used to implement the invention.

Moreover, the invention is not limited to the specific applications described. The system and method of the invention have many other applications both inside and outside the healthcare industry. For example, pharmaceutical manufacturers may apply the system in the clinical development and post marketing surveillance of new drugs, using the system as an interactive, on-line monitoring tool for collecting data on the efficacy, side effects, and quality of life impact of the drugs. Compared to the current use of labor intensive patient interviews, the system provides a fast, flexible, and cost effective alternative for monitoring the use and effects of the drugs.

The system may also be used by home healthcare companies to enhance the service levels provided to customers, e.g. panic systems, sleep surveillance, specific monitoring of disease conditions, etc. Alternatively, the system may be used to monitor and optimize the inventory of home stationed health supplies. As an example, the system may be connected to an appropriate measuring device to optimize timing of oxygen tank delivery to patients with chronic obstructive pulmonary disease (COPD).

It will be clear to one skilled in the art that the above embodiments may be altered in many ways without departing from the scope of the invention. Generally, the dose data may include, for example, quantities of liquid in the syringe before and/or after the

administration of the dose, or dose measurement (capacitive, inductive, or optical) response values before and/or after the administration of the dose. The patient's and/or the clinician's computers then determine the dose administered to the patient from the dose data stored in the recording device. In such an embodiment, calibration data may be stored on the patient's or clinician's computer, and the measurement apparatus may lack a computing device. The patient computer need not be a conventional personal computer, but can be in general any data storage device or device allowing communication between the patient's measurement apparatus and the clinician's data storage device or server. A measurement apparatus of the present invention may connect directly to a clinician's server, rather than indirectly through a patient computer. Various computation and storage devices used in the present invention may generally be implemented through software or dedicated hardware, or combinations thereof. For a multiple-delivery injection device such as an injection pen, liquid quantities before and after each injection are measured and the administered dose is taken to be the difference between the two quantities. The dose measurement aspects of the present invention are not limited to diabetes care, and may be used for monitoring patient compliance with any injection-based treatment program.

Various capacitor geometries and placements may be suitable in a device of the present invention. In particular, the capacitor need not laterally enclose the syringe completely or even partially, as long as the capacitive element is capacitively coupled to the syringe. The method does not require the presence of a plunger to determine capacitance. A method of the present invention may be used to capacitively measure liquid levels in plungerless syringes operated using air pressure, for example.

Similarly, various inductive element geometries and placements may be suitable in a device of the present invention. In particular, the inductive element need not laterally enclose the syringe completely or even partially, as long as the inductive element is inductively coupled to the syringe. The method does not require the presence of a plunger to determine inductance; a method of the present invention may be used to inductively

measure liquid levels in plungerless injection devices operated using air pressure, for example.

With regard to optical based dose measurement embodiments of the invention, detecting spatial distributions is useful for increasing sensitivity. The detector need not detect a spatial distribution of light, however. The detector may detect a spatial sum of light intensity over a whole area, as long as that spatial sum is indicative of the dose administered with the syringe. For example, the detector may detect the total amount of light passing through the syringe, or the total amount of light emitted by the syringe following absorption of incident light (e.g. the total amount of heat emitted following exposure to microwave radiation). Moreover, light emitting and detecting elements need not be longitudinally spaced or aligned, and light beams need not be transverse to the longitudinal axis of the syringe. Various light source and detector geometries and placements may be suitable in a device of the present invention.

An optical method of dose measurement does not require the presence of a plunger to transmit, reflect or absorb light. A method of the present invention may be used to optically measure liquid levels in plungerless syringes operated using air pressure, for example.

The optical dose measurement methods and devices described above may be extended to non-optical wave energy forms such as sound (non-electromagnetic) waves. The considerations discussed above for choosing frequency and detector parameters for optical detectors largely apply to an apparatus using sound wave detection. For example, suitable sound frequencies may include frequencies for which sound absorption by water is significantly (e.g. at least by a factor of two) different from absorption by the syringe plunger. Sound frequencies above the hearing range may be desirable so as to avoid disturbing the user.

Therefore, the scope of the invention should be determined not by the examples given, but by the appended claims and their legal equivalents.

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CLAIMS

What is claimed is:

1. A system for remotely monitoring a dose of a drug administered to a patient, comprising:
   a) a server;
   b) a remote interface means connected to the server;
   c) a measurement apparatus for providing measurement data related to the patient; and
   d) a remotely programmable apparatus, in communication with said measurement apparatus, for receiving measurement data from said measurement apparatus, said remotely programmable apparatus in communication with said server via a communication network; wherein said measurement apparatus provides drug dose measurement data indicative of the dose.

2. The system of claim 1, wherein said measurement apparatus includes a recording device for recording the drug dose measurement data, said recording device in communication with said remotely programmable apparatus.

3. The system of claim 2, wherein said measurement apparatus further includes: a dose measurement element, and a measuring device in communication with said dose measurement element, said measurement apparatus for measuring a response of said dose measurement element, and wherein said measuring device is in communication with said recording device.

4. The system of claim 3, wherein said dose measurement element comprises a capacitive element or an inductive element.

5. The system of claim 2, wherein said measurement apparatus further includes a monitoring device for monitoring a physiological condition of the patient and for generating a condition datum representative of the physiological condition of the patient, said monitoring device in communication with said recording device such that said recording device records said condition datum.

6. The system of claim 5, wherein said monitoring device comprises a blood glucose meter and said condition datum comprises a blood glucose level of the patient.

7. The system of claim 2, wherein said recording device comprises a digital memory unit.

8. The system of claim 3, wherein said measurement apparatus further includes:

a display connected to said measuring device, said display for displaying the dose; and

a computing device in communication with said recording device, said computing device for computing said drug dose measurement data from the response of said dose measurement element.

9. The system of claim 8, wherein said measurement apparatus further includes a calibration memory in communication with said computing device, said calibration memory for providing said computing device with calibration data indicative of a correspondence between the response of said dose measurement element and said drug dose measurement data.

10. The system of claim 3, wherein said measurement apparatus further includes a holder for receiving and holding a syringe in a measurement position.

11. The system of claim 10, further comprising a housing enclosing said measuring device and said recording device, wherein said holder is mechanically coupled to said housing.

12. The system of claim 11, wherein said housing further encloses said holder and said dose measurement element, said housing shielding said dose measurement element and the syringe from external electric fields.

13. The system of claim 10, wherein said holder comprises a well, said well laterally enclosing the syringe in the measurement position, and said dose measurement element is coupled to said well so as to laterally enclose the syringe in the measurement position.

14. The system of claim 10, wherein said dose measurement element comprises a capacitive element, said capacitive element being defined between a liquid held by the syringe and an electrode located external to the syringe, wherein said measurement apparatus further comprises a needle contact coupled to said holder, said needle contact for establishing electrical communication between said measuring device and a needle of the syringe when the syringe is in the measurement position, and said needle is in electrical contact with the liquid.

15. The system of claim 10, wherein said dose measurement element comprises a capacitive element, said capacitive element including:

a) a first electrically conducting longitudinal plate coupled to said holder; and

b) a second electrically conducting longitudinal plate coupled to said holder opposite said first longitudinal plate, and said second plate electrically insulated from said first plate.

16. The system of claim 10, wherein said inductive element is coupled to said holder such that an inductive response of said inductive element is indicative of the dose when the syringe is in the measurement position.

17. The system of claim 1, wherein the measurement apparatus further includes:

a) a syringe having a barrel and an inductance-enhancing element; and

b) a holder for receiving and holding said syringe in a measurement position, wherein a position of said inductance-enhancing element relative to said barrel of said syringe is indicative of the dose, and wherein said inductive response is indicative of said position of said inductance-enhancing element.

18. The system of claim 17, wherein said syringe includes a plunger, and said inductance-enhancing element comprises a ferromagnetic material, a longitudinal plunger element, a ferromagnetic plunger core, or a plurality of distinct sections arranged longitudinally within said plunger.

19. The system of claim 1, wherein the measurement apparatus further includes:

a) a holding means for receiving and holding a syringe in a measurement position;

b) a light source coupled to said holding means and in optical communication with the syringe, for generating light incident on the syringe when said syringe is in the measurement position, wherein an optical response of the syringe to said light is indicative of said dose; and

c) an optical detector coupled to said holding means and in optical communication with the syringe, for detecting the optical response; wherein said optical detector is in electrical communication with said recording device, said recording device for recording a dose datum indicative of said optical response, wherein said dose datum is indicative of the dose.

20. The system of claim 1, wherein said server includes a script generating means for generating a script program executable by said remotely programmable apparatus to communicate a message to the patient.

21. The system of claim 20, wherein the remotely programmable apparatus comprises:

i) a communication means for transmitting the measurement data to said server;

ii) a memory means for storing the script program;

iii) a user interface means for communicating the message to the patient; and

iv) a processor means connected to said communication means, said user interface means, and said memory means for executing the script program.

22. The system of claim 20, wherein said remotely programmable apparatus includes:

i) communication means for receiving the script program from said server and for transmitting to said server responses to the queries;

ii) user interface means for communicating queries to the patient and for receiving responses to the queries;

iii) memory means for storing the script program and responses to the queries;

iv) processor means connected to said communication means, said user interface means, and said memory means for executing the script program to communicate the queries to the patient, to receive the responses to the queries, and to transmit the responses to said server; and

v) device interface means connected to said processor means for receiving the measurement data from said measurement apparatus; wherein said memory means includes means for storing the measurement data, and said communication means includes means for transmitting the measurement data to said server.

23. A system for remotely monitoring a dose of a drug administered to a patient, comprising:

a) a server;

b) a remote interface means connected to the server;

c) a measurement apparatus for providing measurement data related to the patient; and

d) a remotely programmable apparatus for receiving measurement data from the measurement apparatus, the remotely programmable apparatus being

networked to the server via a communication network; wherein the measurement apparatus includes:

i) a syringe having a barrel, a plunger, and a response-enhancing element;

ii) a holding means for receiving and holding said syringe in a measurement position;

iii) a light source coupled to said holding means and in optical communication with said syringe, for generating light incident on said syringe, wherein an optical response of said syringe to said light is indicative of said dose; and

iv) an optical detector coupled to said holding means and in optical communication with said syringe, for detecting the optical response;

v) a recording device in electrical communication with said optical detector; and

vi) an alignment means for aligning said barrel of said syringe to said optical detector, said recording device for recording a dose datum indicative of said optical response, wherein said dose datum is indicative of the dose.

24. The system of claim 23, wherein a position of a marking of said response-enhancing element is indicative of said dose; and said optical response depends on an interaction of the light with said marking in said position.

25. The system of claim 23, wherein said response-enhancing element comprises a longitudinal element mechanically coupled to said plunger, and said longitudinal element is longitudinally marked.

26. The system of claim 24, wherein said marking comprises a longitudinally-variable color marking which varies longitudinally in brightness or in hue.

27. The system of claim 23, wherein said optical detector is situated opposite said light source relative to said syringe, or adjacent said light source relative to said syringe.

28. A system for monitoring a patient, comprising:

a) a server;

b) a remote interface means connected to the server;

c) a remotely programmable apparatus for receiving measurement data from the measurement apparatus, the remotely programmable apparatus being networked to the server via a communication network; and

d) an optical dose measurement apparatus, in communication with said server, for providing a dose datum of a drug delivered to the patient from a syringe; wherein the optical dose measurement apparatus includes:

i) a holding means for receiving and holding the syringe;

ii) at least one optical detecting element coupled to said holding means and in optical communication with the syringe, for detecting an optical response pattern of the syringe, wherein said optical response pattern is indicative of the dose; and

iii) a recording means in electrical communication with said at least one optical detecting element, for recording a dose datum indicative of said optical response pattern, wherein said dose datum is indicative of the dose.

29. A method for remotely monitoring a patient, comprising the steps of:

a) providing the patient with a remotely programmable apparatus and a measurement apparatus in communication with the remotely programmable apparatus, the measurement apparatus having:

i) a dose measurement element,

ii) a measuring device in communication with the dose measurement element for measuring a response of the dose measurement element; and

iii) a recording device in communication with the measuring device, wherein the measurement apparatus provides measurement data related to the patient;

b) collecting the measurement data in the remotely programmable apparatus;

c) transmitting the measurement data from the remotely programmable apparatus to a server; and

d) receiving and storing the measurement data in the server.

30. The method of claim 29, wherein the remotely programmable apparatus includes:

i) a communication means for exchanging data between the remotely programmable apparatus and the server through a communication network;

ii) a memory means for storing the data exchangeable between the remotely programmable apparatus and the server; and

iii) a processor means connected to the communication means, and the memory means.

31. The method of claim 29, wherein the measurement data related to the patient comprises dose measurement data indicative of a dose of a drug delivered to the patient.

32. The method of claim 29, wherein the dose measurement element comprises a capacitive element or an inductive element.

33. The method of claim 33, wherein the measurement apparatus further includes a recording device and a monitoring device, wherein the monitoring device provides physiological condition measurements to the recording device, the physiological condition measurements indicative of a physiological condition of the patient; wherein the remotely programmable apparatus further includes a device interface connected to the processor means for receiving the physiological condition

measurements; and wherein said step b) comprises collecting the physiological condition measurements in the remotely programmable apparatus.

34. The method of claim 33, wherein the physiological condition comprises diabetes, and the physiological condition measurements comprise a blood glucose level of the patient.

35. The method of claim 30, wherein the data exchangeable between the remotely programmable apparatus and the server includes a script program, executable by the remotely programmable apparatus, to communicate queries to the patient, to receive responses to the queries, and to transmit the responses to the server; and wherein the remotely programmable apparatus further includes a user interface means for communicating the queries to the patient and for receiving the responses to the queries.

36. The method of claim 35, wherein the server comprises a web server having a web page for entry of the queries, and wherein the queries are entered by accessing the web page through the Internet and entering the queries in the web page.

37. A method for communicating information to a patient, the method comprising the steps of:

a) providing the patient with a measurement apparatus and a remotely programmable apparatus in communication with the measurement apparatus, the remotely programmable apparatus having:

i) a communication means for exchanging data with a server through a communication network, wherein the exchangeable data includes a script program executable by the remotely programmable apparatus to communicate a message to the patient;

ii) a memory means for storing the script program;

iii) a user interface for communicating the message; and

i) a processor means connected to the communication means, the memory means, and the user interface for executing the script program;

and the measurement apparatus having:

vi) a measuring device;

vii) and a recording device in communication with the measuring device and the remotely programmable apparatus;

b) generating measurement data from the measurement apparatus related to the patient;

c) transmitting the measurement data from the recording device to the remotely programmable apparatus via a standard cable; and

d) transmitting the measurement data from the remotely programmable apparatus to the server through the communication network.

38. The method of claim 37, wherein the measurement apparatus further includes a dose measurement element, the measuring element for measuring a response of the dose measurement element.

39. The method of claim 38, wherein the measurement apparatus further includes a monitoring device for monitoring a physiological condition of the patient, and wherein the measurement data transmitted in said steps c) and d) includes physiological condition data and dose measurement data.

40. The method of claim 39, wherein the physiological condition data comprises a blood glucose level of a patient, and the dose measurement data comprises a dose of insulin administered to the patient.

41. The method of claim 39, wherein the dose measurement data comprises a dose of a drug administered to the patient from a syringe.

42. The method of claim 41, wherein the syringe includes a plunger, a barrel, and an inductance-enhancing element, wherein a position of the inductance-enhancing element relative to the barrel is indicative of the dose, and the inductance-enhancing element comprises a ferromagnetic material, a longitudinal plunger element, a ferromagnetic plunger core, or a plurality of distinct sections arranged longitudinally within the plunger.

43. The method of claim 41, wherein the measurement apparatus further includes:

i) a holder for receiving and holding the syringe in a measurement position; and

ii) a capacitive element coupled to the holder such that a capacitive response of the capacitive element is indicative of the dose when the syringe is in the measurement position; wherein the measuring device is in communication with the capacitive element for measuring the capacitive response; and wherein the recording device is in communication with the measuring device for recording a dose datum indicative of the capacitive response, wherein the dose datum is indicative of the dose.

44. The method of claim 37, wherein the measurement apparatus further includes:

i) a holder for receiving and holding a syringe in a measurement position,

ii) a light source in optical communication with the syringe when the syringe is in the measurement position; and

iii) an optical detector in optical communication with the syringe when the syringe is in the measurement position, the optical detector for detecting an optical response; and the syringe includes a response-enhancing element having a marking, wherein a position of the marking of the response-enhancing element is indicative of the dose.

45. The method of claim 37, further comprising the steps of:

e) entering in the server the message to be communicated to the patient;

f) generating the script program in the server;

g) transmitting the script program from the server to the remotely programmable apparatus through the communication network; and

h) executing the script program in the apparatus to communicate the message to the patient.

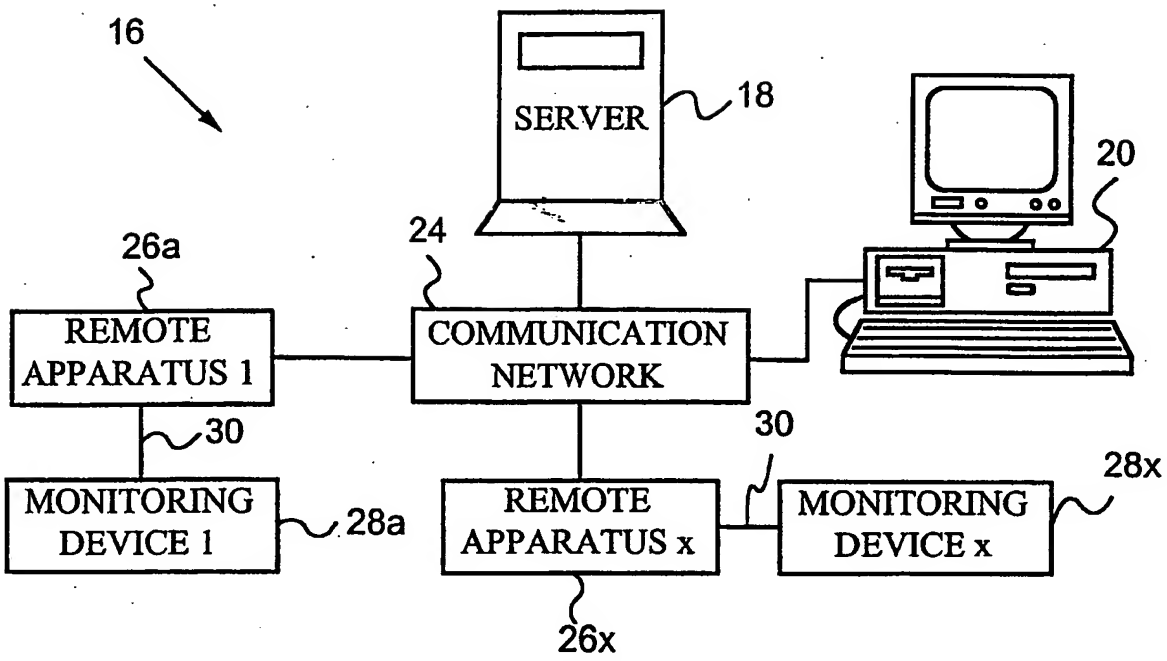

16
SERVER
18
20
24
26a
REMOTE APPARATUS 1
COMMUNICATION NETWORK
30
30
28x
MONITORING DEVICE 1
28a
REMOTE APPARATUS x
MONITORING DEVICE x
26x

FIG. 1

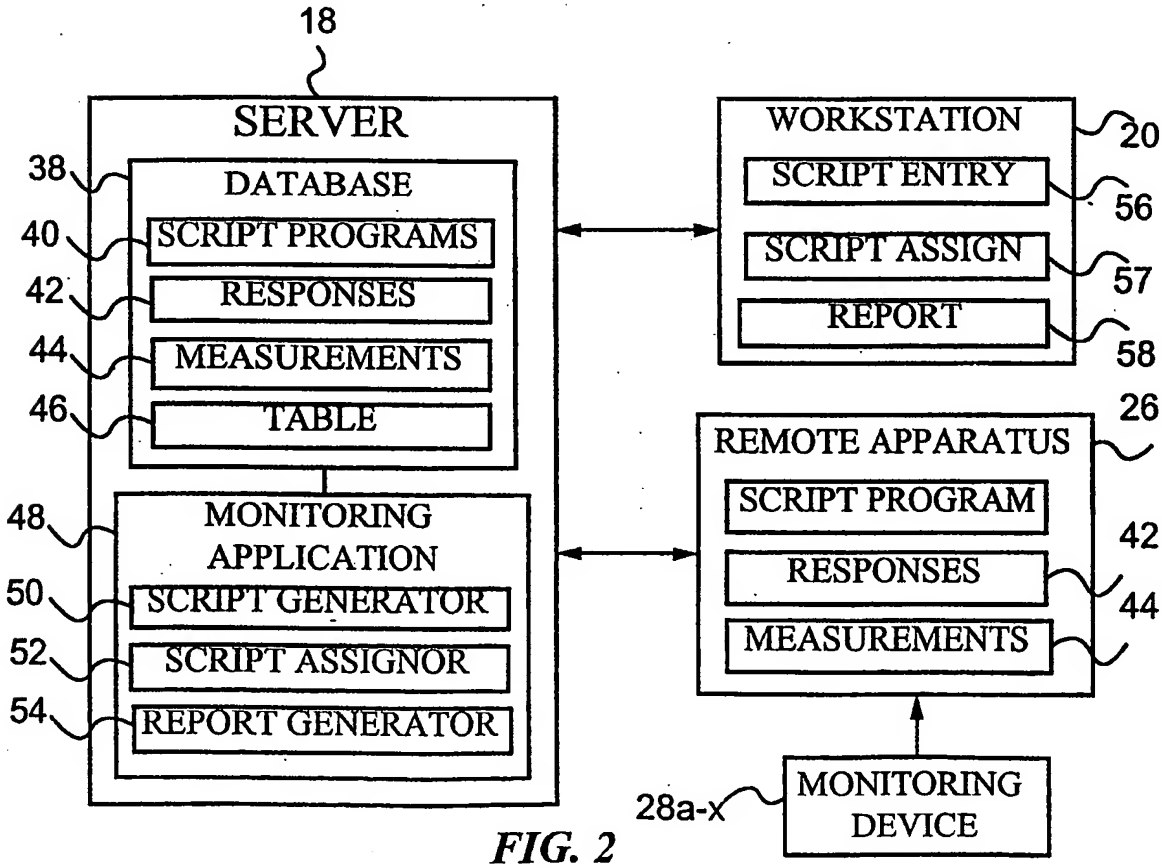

18
SERVER
38
DATABASE
40
SCRIPT PROGRAMS
42
RESPONSES
44
MEASUREMENTS
46
TABLE
48
MONITORING APPLICATION
50
SCRIPT GENERATOR
52
SCRIPT ASSIGNOR
54
REPORT GENERATOR
WORKSTATION
20
SCRIPT ENTRY
56
SCRIPT ASSIGN
57
REPORT
58
26
REMOTE APPARATUS
SCRIPT PROGRAM
42
RESPONSES
44
MEASUREMENTS
28a-x
MONITORING DEVICE

FIG. 2

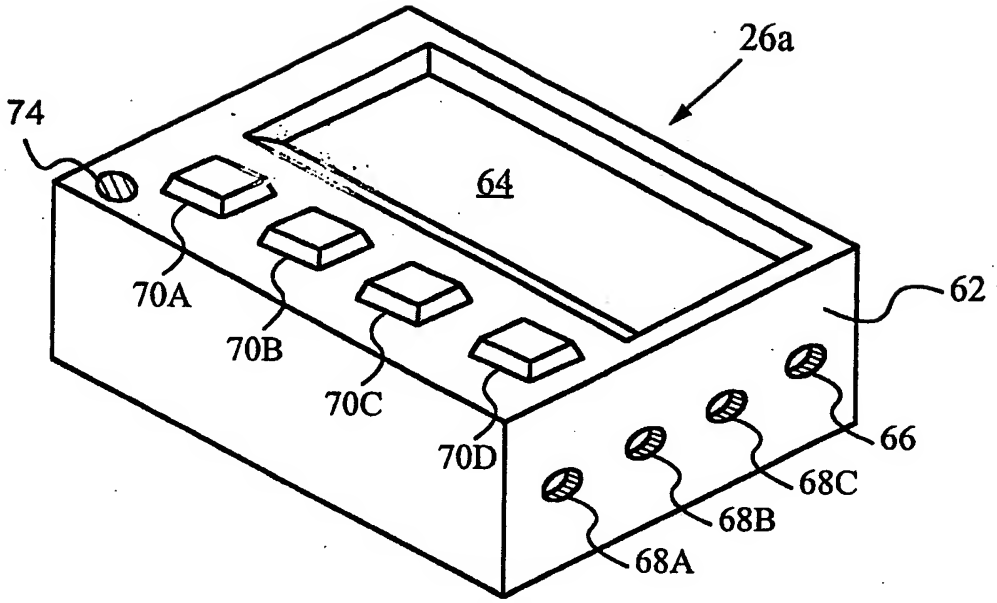

26a
74
64
70A
70B
70C
70D
62
66
68C
68B
68A

FIG. 3

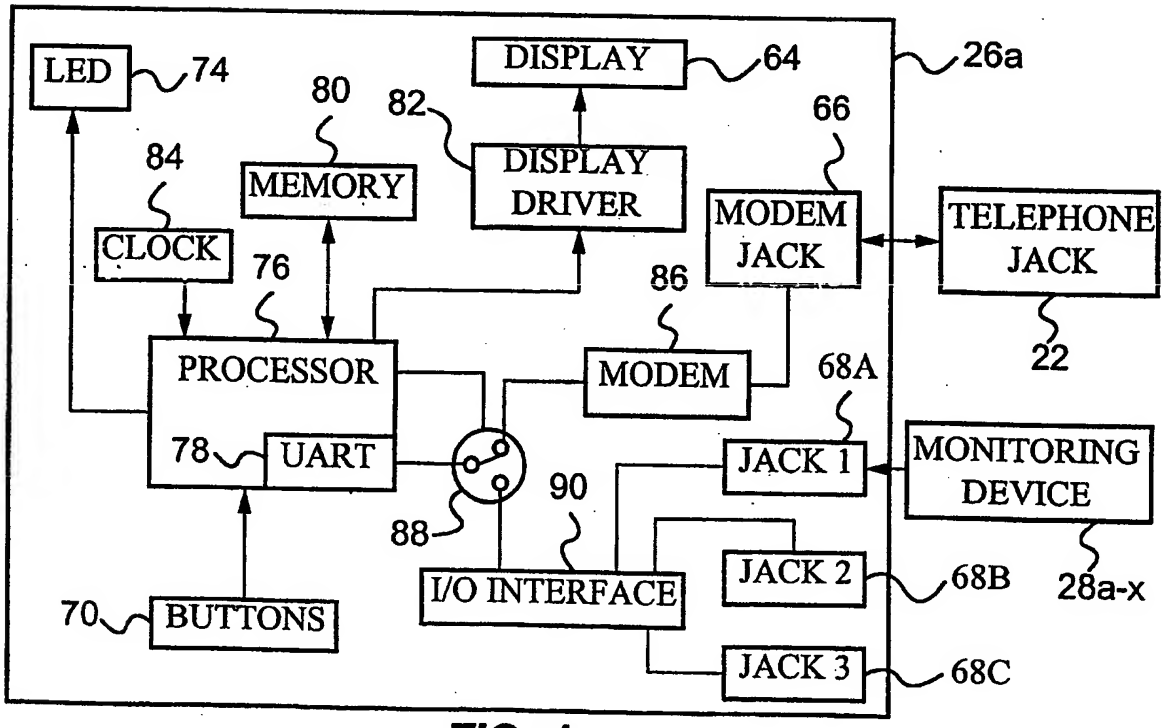

LED
74
DISPLAY
64
26a
80
82
66
84
MEMORY
DISPLAY DRIVER
MODEM JACK
TELEPHONE JACK
CLOCK
76
86
22
PROCESSOR
MODEM
68A
78
UART
JACK 1
MONITORING DEVICE
90
88
JACK 2
68B
28a-x
70
BUTTONS
I/O INTERFACE
JACK 3
68C

FIG. 4

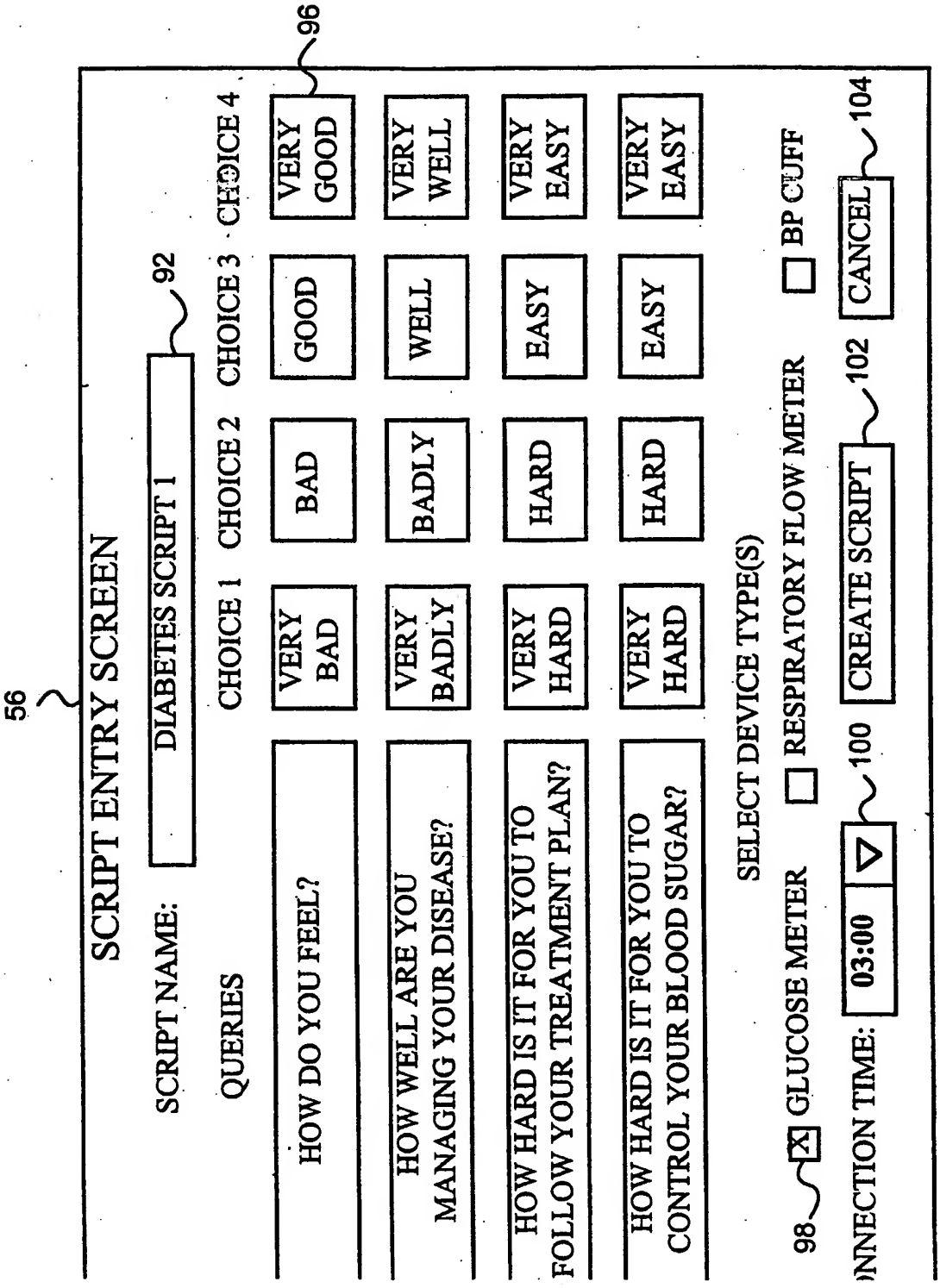

56
SCRIPT ENTRY SCREEN
SCRIPT NAME:
DIABETES SCRIPT 1
92
QUERIES
CHOICE 1
CHOICE 2
CHOICE 3
CHOICE 4
96
HOW DO YOU FEEL?
VERY BAD
BAD
GOOD
VERY GOOD
HOW WELL ARE YOU MANAGING YOUR DISEASE?
VERY BADLY
BADLY
WELL
VERY WELL
HOW HARD IS IT FOR YOU TO FOLLOW YOUR TREATMENT PLAN?
VERY HARD
HARD
EASY
VERY EASY
HOW HARD IS IT FOR YOU TO CONTROL YOUR BLOOD SUGAR?
VERY HARD
HARD
EASY
VERY EASY
SELECT DEVICE TYPE(S)
98
GLUCOSE METER
RESPIRATORY FLOW METER
BP CUFF
NNECTION TIME:
03:00
100
CREATE SCRIPT
102
CANCEL
104

FIG. 5

NUMBER: 9001 {LF}

LED: 1 {LF}

ZAP: {LF}

CLS: {LF}

DISPLAY: ANSWER QUERIES NOW?
PRESS ANY BUTTON TO START {LF}
WAIT: {LF}

CLS: {LF}

DISPLAY: HOW DO YOU FEEL?

VERY VERY
BAD BAD GOOD GOOD {LF}
INPUT: OOOO {LF}

CLS: {LF}

DISPLAY: HOW WELL ARE YOU
MANAGING YOUR DISEASE?
VERY VERY
WELL BADLY WELL WELL {LF}
INPUT: OOOO {LF}

CLS: {LF}

DISPLAY: HOW HARD IS IT FOR YOU TO
FOLLOW YOUR TREATMENT PLAN?
VERY VERY
HARD HARD EASY EASY {LF}
INPUT: OOOO {LF}

CLS: {LF}

DISPLAY: HOW HARD IS IT FOR YOU TO
CONTROL YOUR BLOOD SUGAR?
VERY VERY
HARD HARD EASY EASY {LF}

FIG. 6A

INPUT: OOOO {LF}

CLS: {LF}

DISPLAY: CONNECT GLUCOSE METER
AND PRESS ANY BUTTON
WHEN FINISHED {LF}

WAIT: {LF}

CLS: {LF}

DISPLAY: COLLECTING MEASUREMENTS {LF}

COLLECT: GLUCOSE_METER {LF}

CLS: {LF}

DISPLAY: CONNECT APPARATUS TO
TELEPHONE JACK AND
PRESS ANY BUTTON
WHEN FINISHED {LF}

WAIT: {LF}

LED: 0 {LF}

CLS: {LF}

DELAY: 03:00 {LF}

DISPLAY: CONNECTING TO SERVER {LF}

CONNECT: {LF}

{EOF}

FIG. 6B

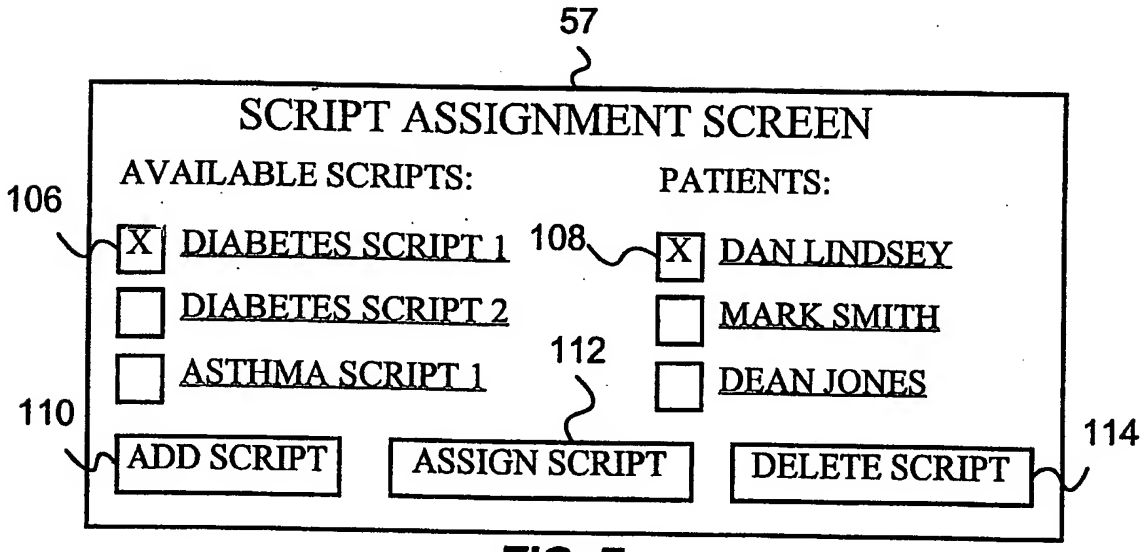


FIG. 7

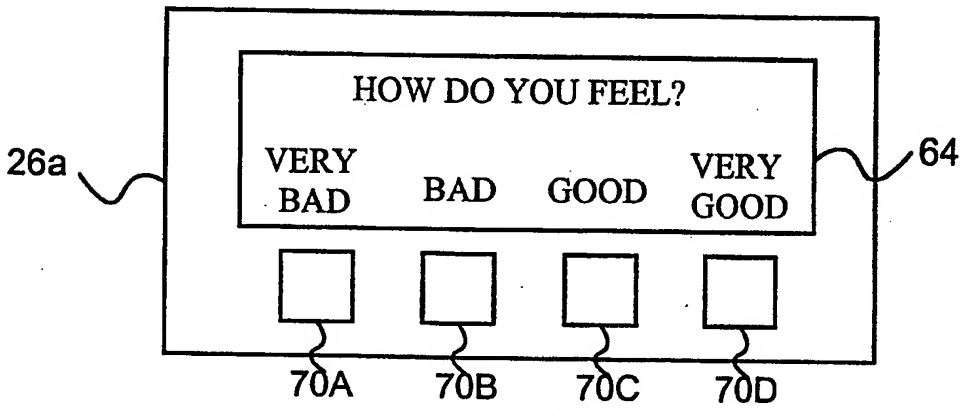


FIG. 8

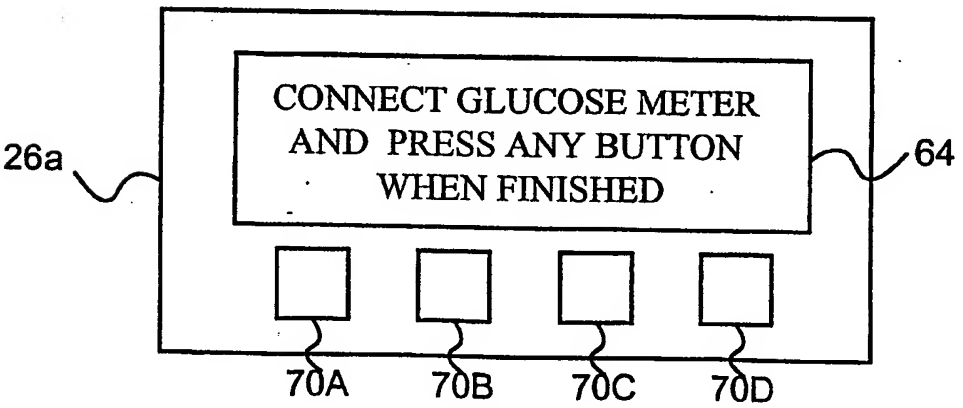


FIG. 9

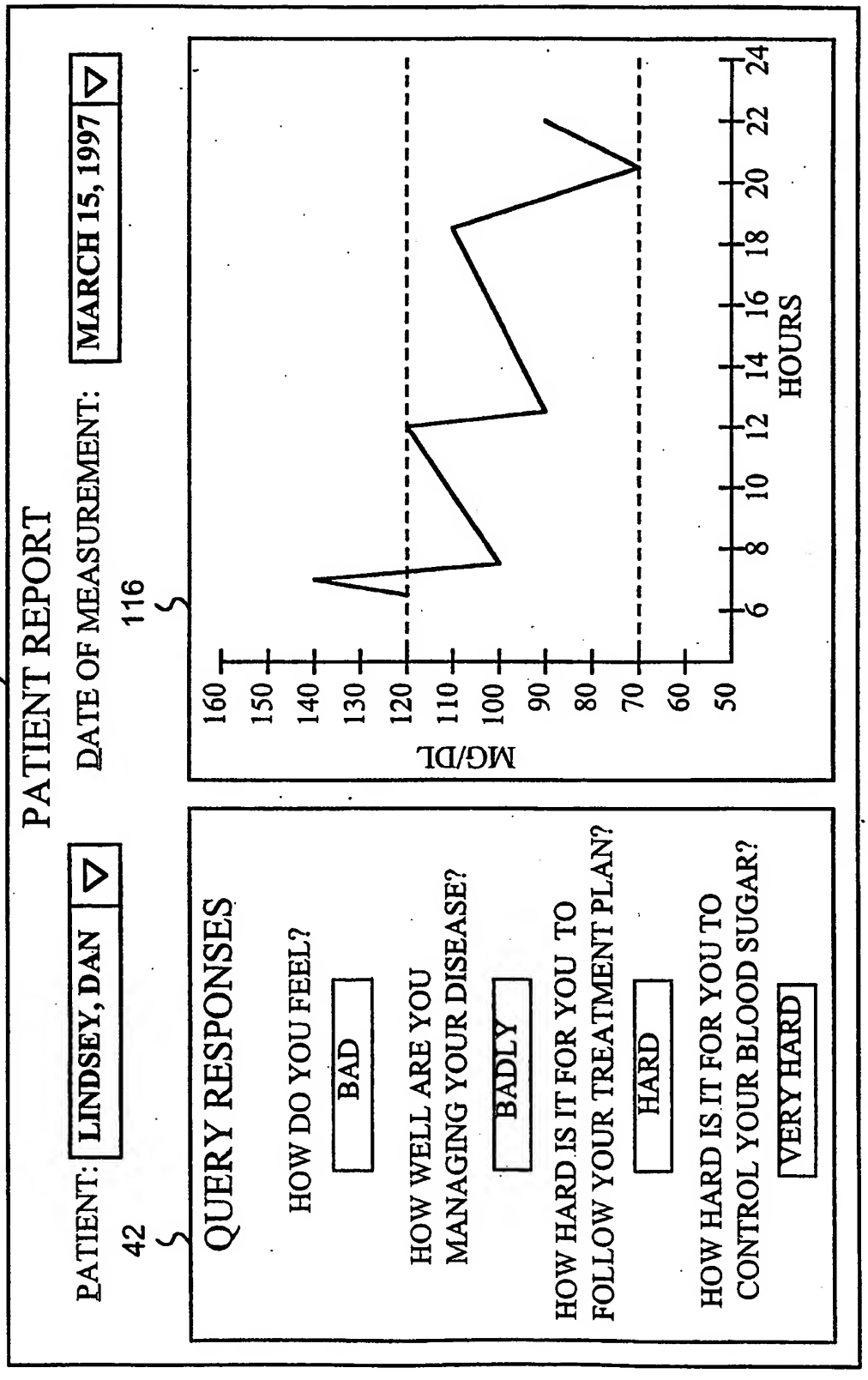

58
PATIENT REPORT
PATIENT: LINDSEY, DAN
DATE OF MEASUREMENT: MARCH 15, 1997
42
116
QUERY RESPONSES
HOW DO YOU FEEL?
BAD
HOW WELL ARE YOU MANAGING YOUR DISEASE?
BADLY
HOW HARD IS IT FOR YOU TO FOLLOW YOUR TREATMENT PLAN?
HARD
HOW HARD IS IT FOR YOU TO CONTROL YOUR BLOOD SUGAR?
VERY HARD
MG/DL
160
150
140
130
120
110
100
90
80
70
60
50
6
8
10
12
14
16
18
20
22
24
HOURS

FIG. 10

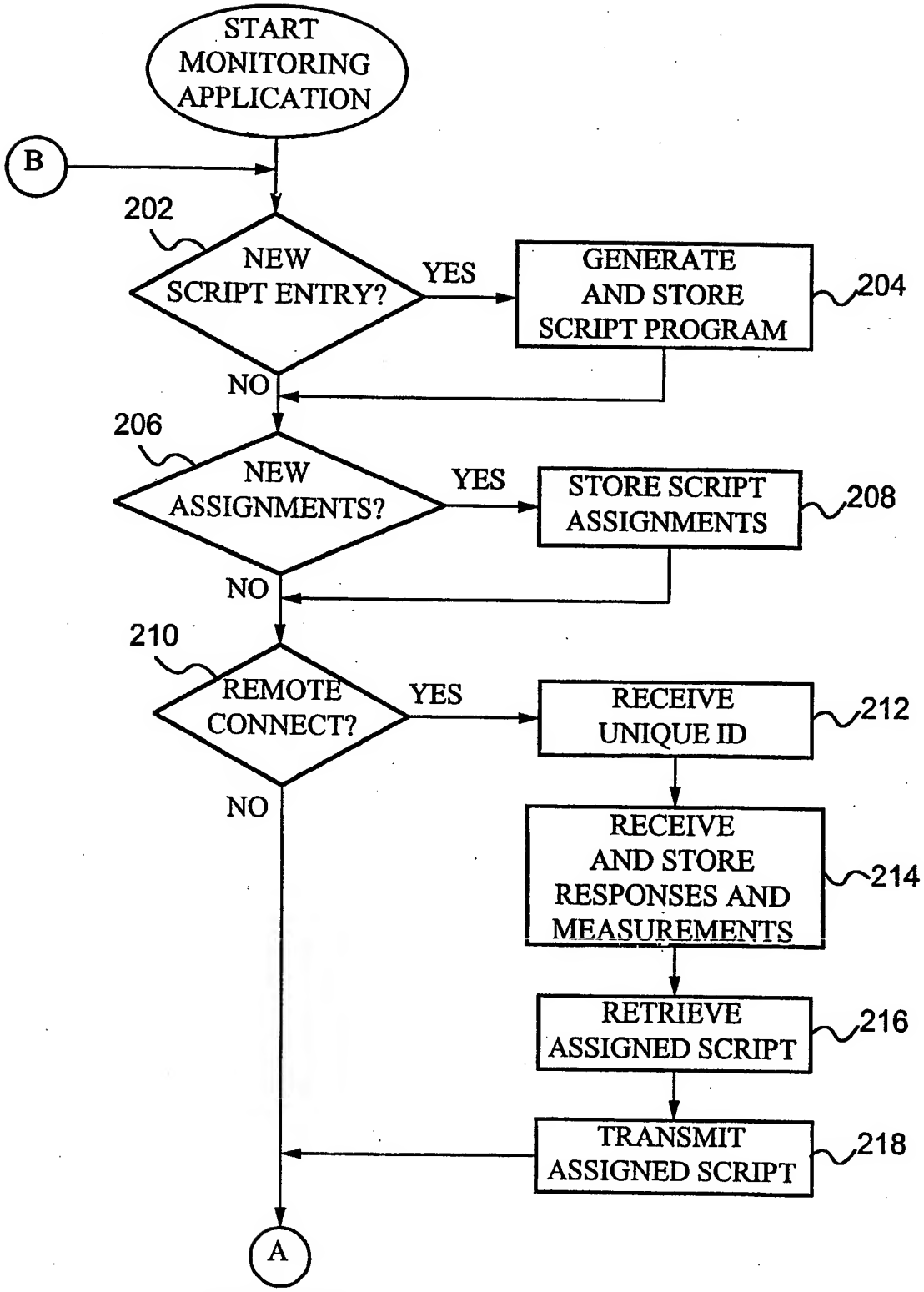

START MONITORING APPLICATION
B
202
NEW SCRIPT ENTRY?
YES
GENERATE AND STORE SCRIPT PROGRAM
204
NO
206
NEW ASSIGNMENTS?
YES
STORE SCRIPT ASSIGNMENTS
208
NO
210
REMOTE CONNECT?
YES
RECEIVE UNIQUE ID
212
NO
RECEIVE AND STORE RESPONSES AND MEASUREMENTS
214
RETRIEVE ASSIGNED SCRIPT
216
TRANSMIT ASSIGNED SCRIPT
218
A

FIG. 11A

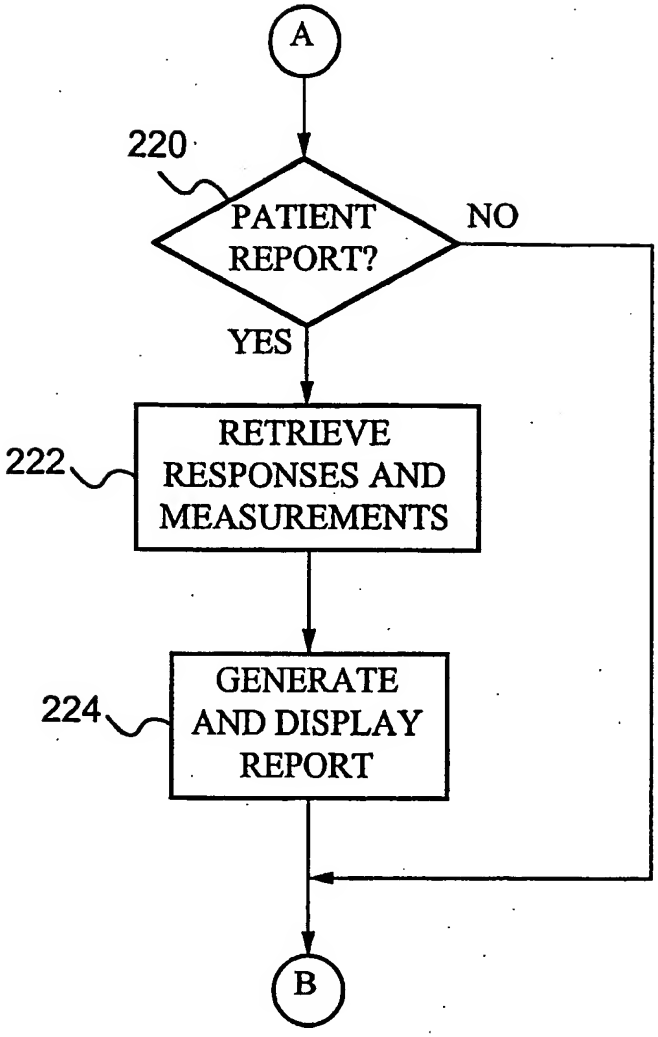

A
220
PATIENT REPORT?
NO
YES
222
RETRIEVE RESPONSES AND MEASUREMENTS
224
GENERATE AND DISPLAY REPORT
B

FIG. 11B

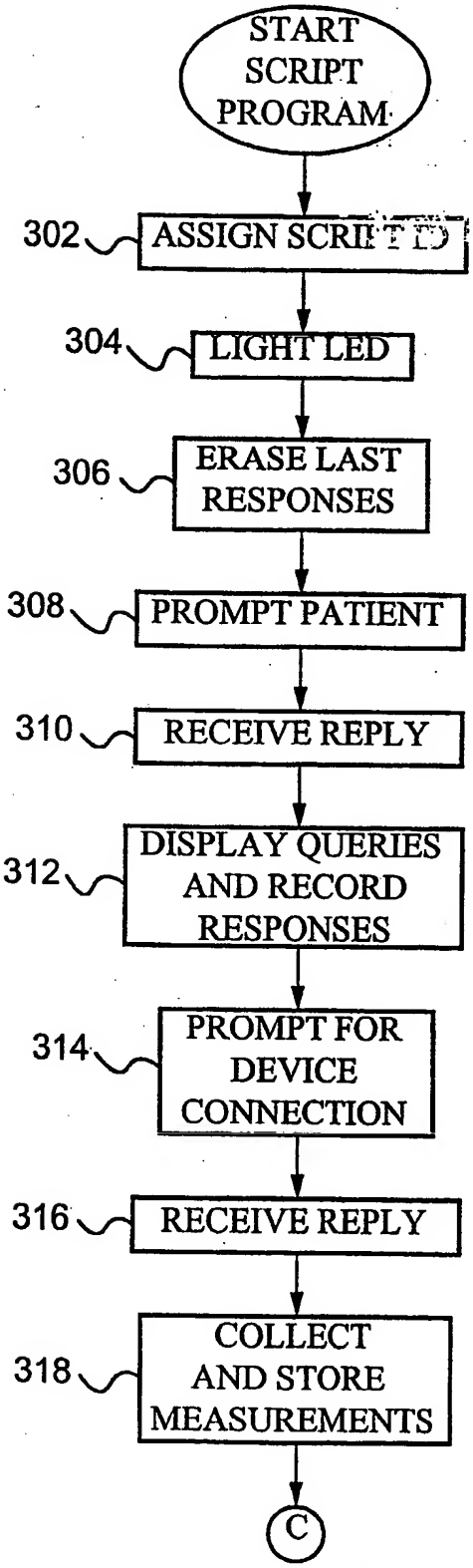

START SCRIPT PROGRAM
302
ASSIGN SCRIPT ID
304
LIGHT LED
306
ERASE LAST RESPONSES
308
PROMPT PATIENT
310
RECEIVE REPLY
312
DISPLAY QUERIES AND RECORD RESPONSES
314
PROMPT FOR DEVICE CONNECTION
316
RECEIVE REPLY
318
COLLECT AND STORE MEASUREMENTS
C

FIG. 12A

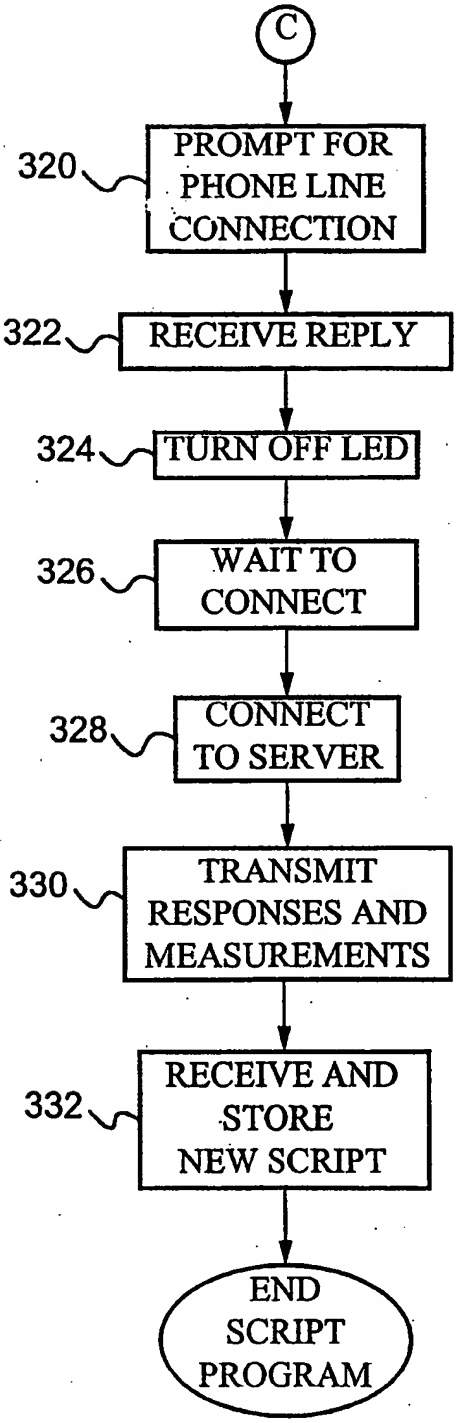

C
320
PROMPT FOR PHONE LINE CONNECTION
322
RECEIVE REPLY
324
TURN OFF LED
326
WAIT TO CONNECT
328
CONNECT TO SERVER
330
TRANSMIT RESPONSES AND MEASUREMENTS
332
RECEIVE AND STORE NEW SCRIPT
END SCRIPT PROGRAM

FIG. 12B

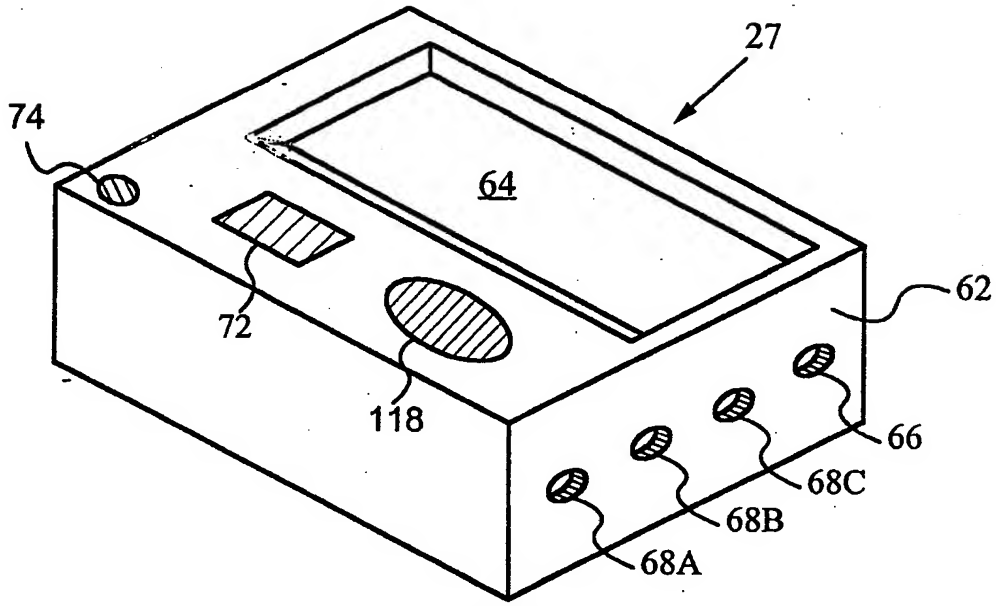

27
74
64
72
118
62
66
68C
68B
68A

FIG. 13

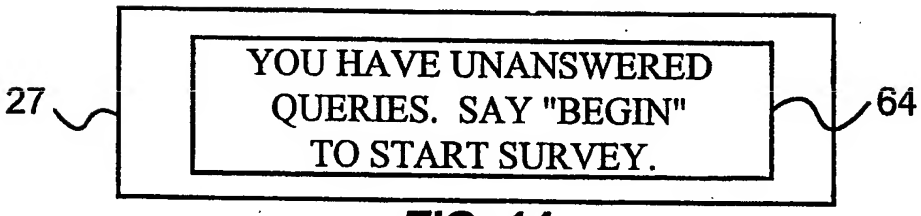

27
YOU HAVE UNANSWERED QUERIES. SAY "BEGIN" TO START SURVEY.
64

FIG. 14

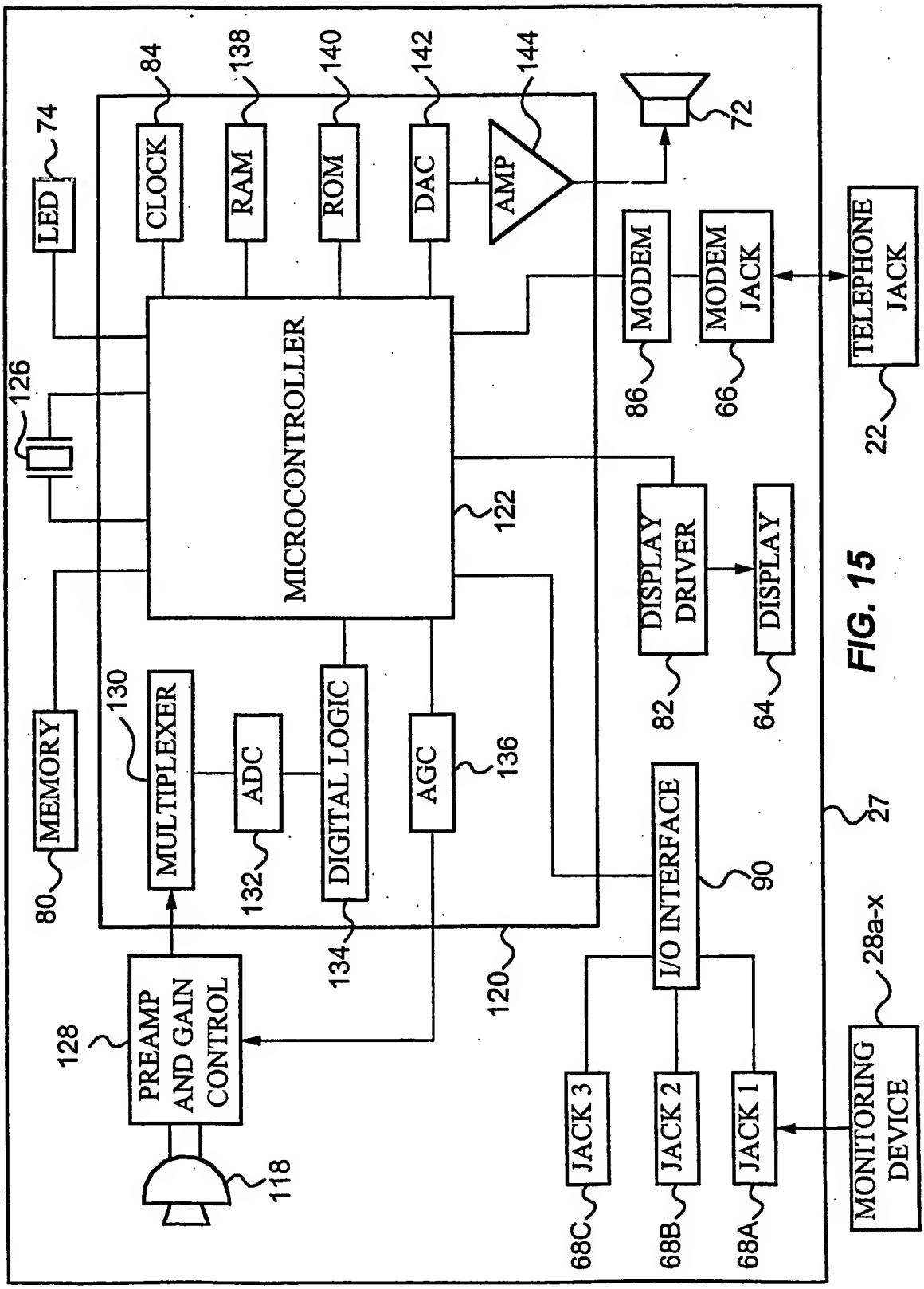

LED
74
CLOCK
84
RAM
138
ROM
140
DAC
142
AMP
144
72
MODEM
86
MODEM JACK
66
TELEPHONE JACK
22
126
MICROCONTROLLER
122
DISPLAY DRIVER
82
DISPLAY
64
MEMORY
80
MULTIPLEXER
130
ADC
132
DIGITAL LOGIC
134
AGC
136
I/O INTERFACE
90
27
PREAMP AND GAIN CONTROL
128
118
120
JACK 3
68C
JACK 2
68B
JACK 1
68A
28a-x
MONITORING DEVICE

FIG. 15

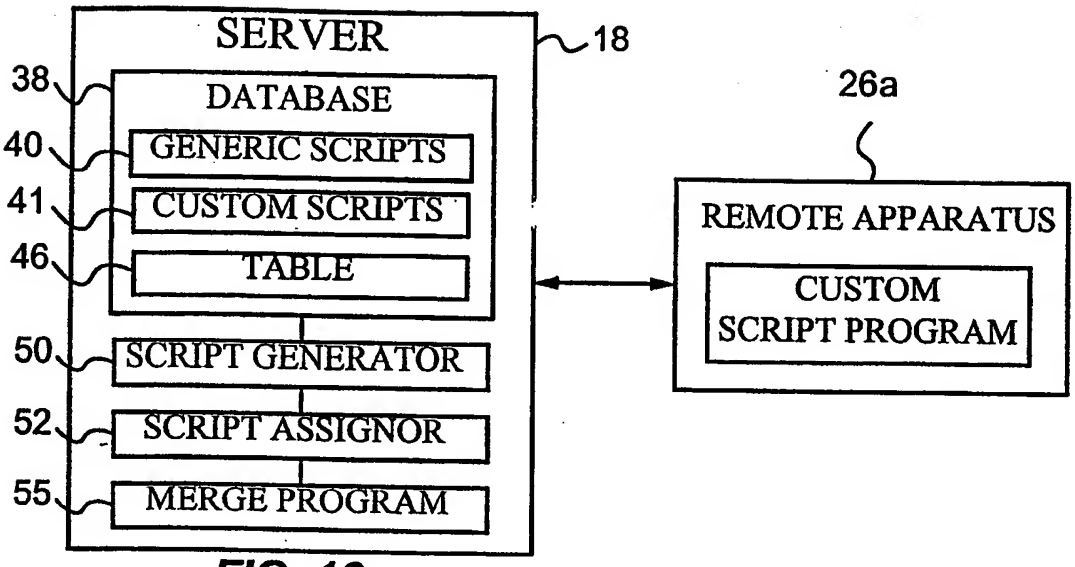


FIG. 16

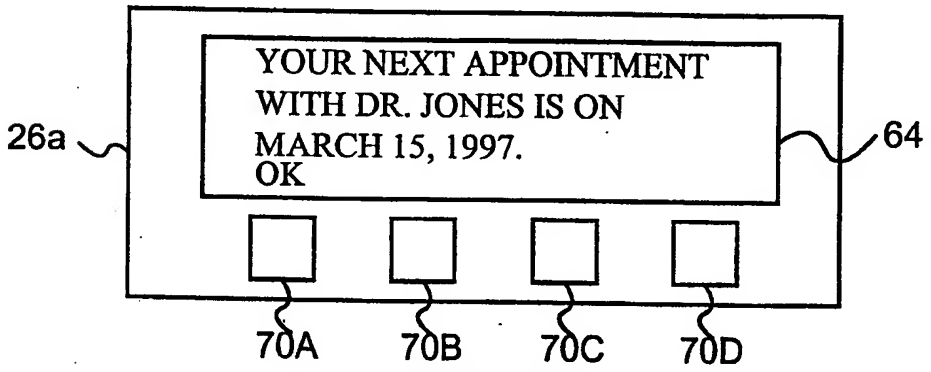


FIG. 17

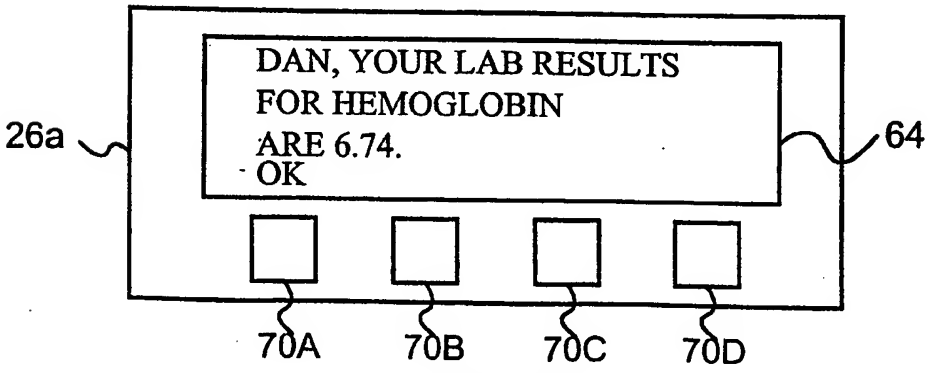


FIG. 18

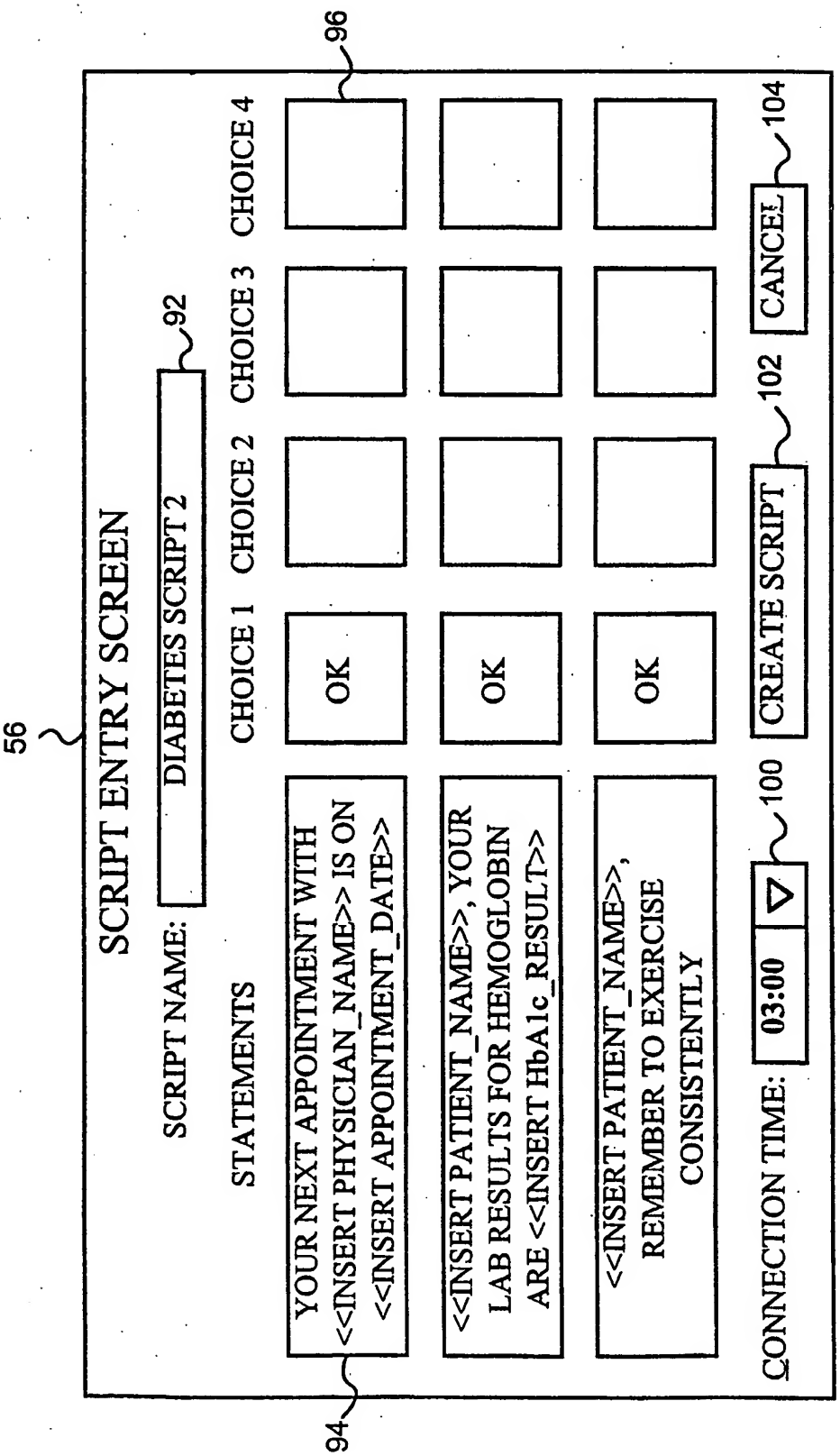

56
SCRIPT ENTRY SCREEN
SCRIPT NAME:
DIABETES SCRIPT 2
92
STATEMENTS
CHOICE 1
CHOICE 2
CHOICE 3
CHOICE 4
96
YOUR NEXT APPOINTMENT WITH <<INSERT PHYSICIAN_NAME>> IS ON <<INSERT APPOINTMENT_DATE>>
OK
<<INSERT PATIENT_NAME>>, YOUR LAB RESULTS FOR HEMOGLOBIN ARE <<INSERT HbA1c_RESULT>>
OK
<<INSERT PATIENT_NAME>>, REMEMBER TO EXERCISE CONSISTENTLY
OK
94
CONNECTION TIME:
03:00
100
CREATE SCRIPT
102
CANCEL
104

FIG. 19

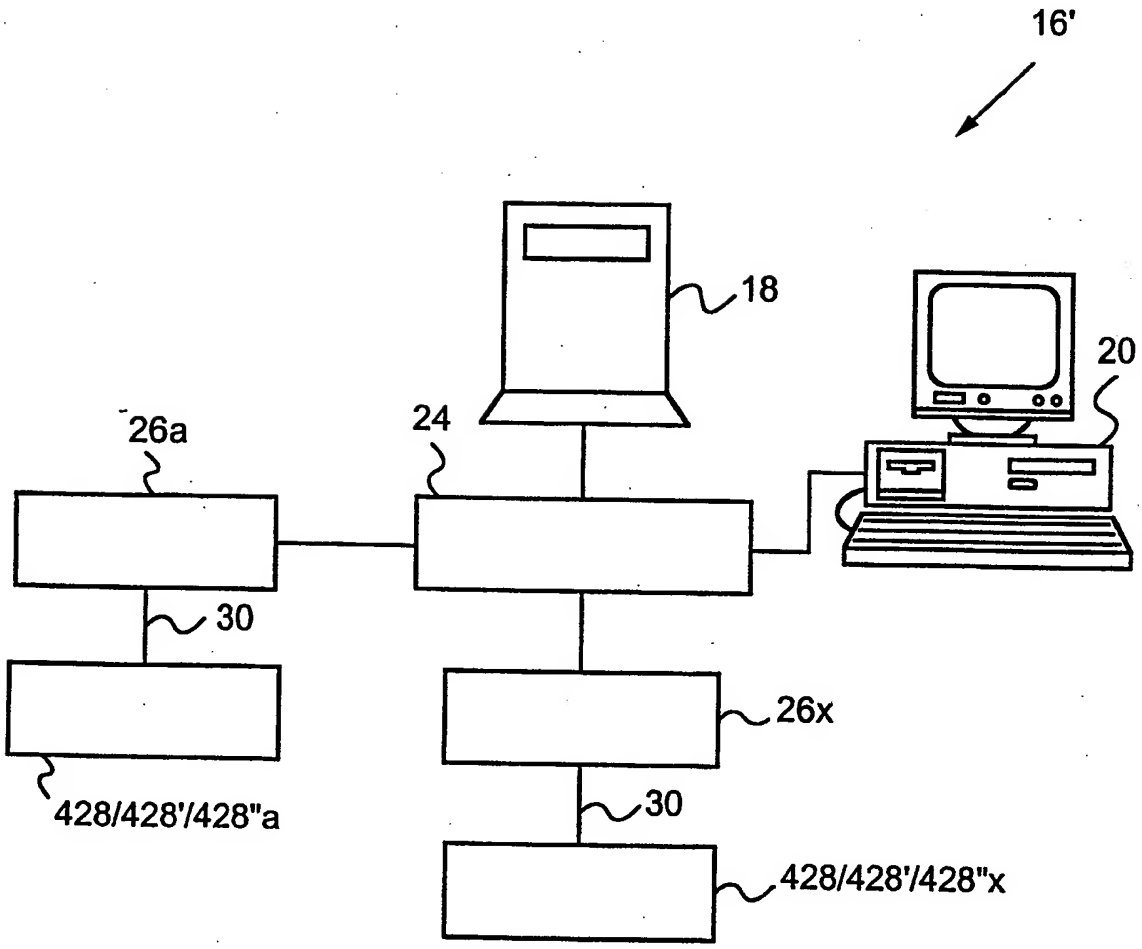


FIG. 20A

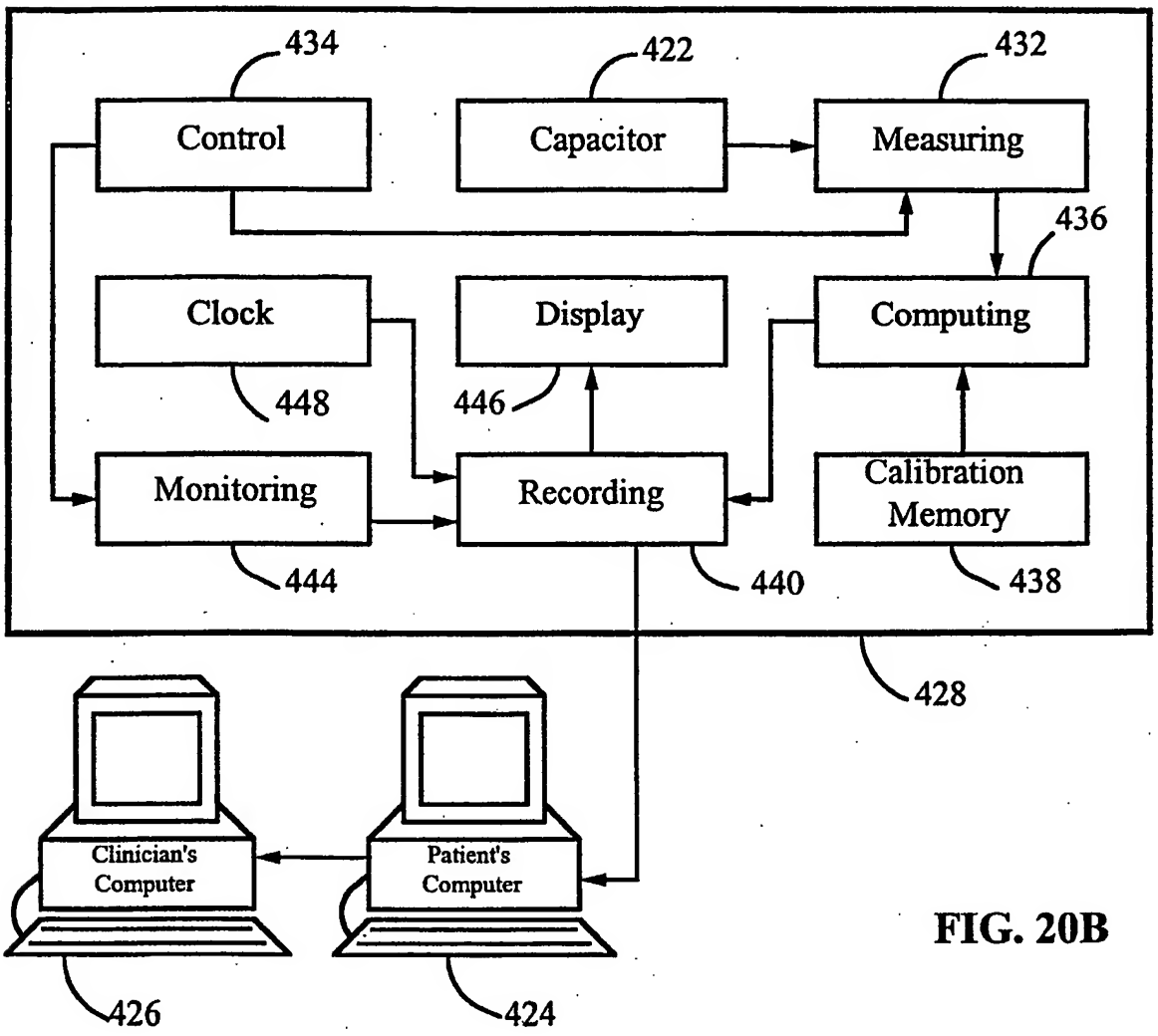

434
422
432
Control
Capacitor
Measuring
436
Clock
Display
Computing
448
446
Monitoring
Recording
Calibration Memory
444
440
438
428
Clinician's Computer
Patient's Computer
426
424

FIG. 20B

FIG. 21

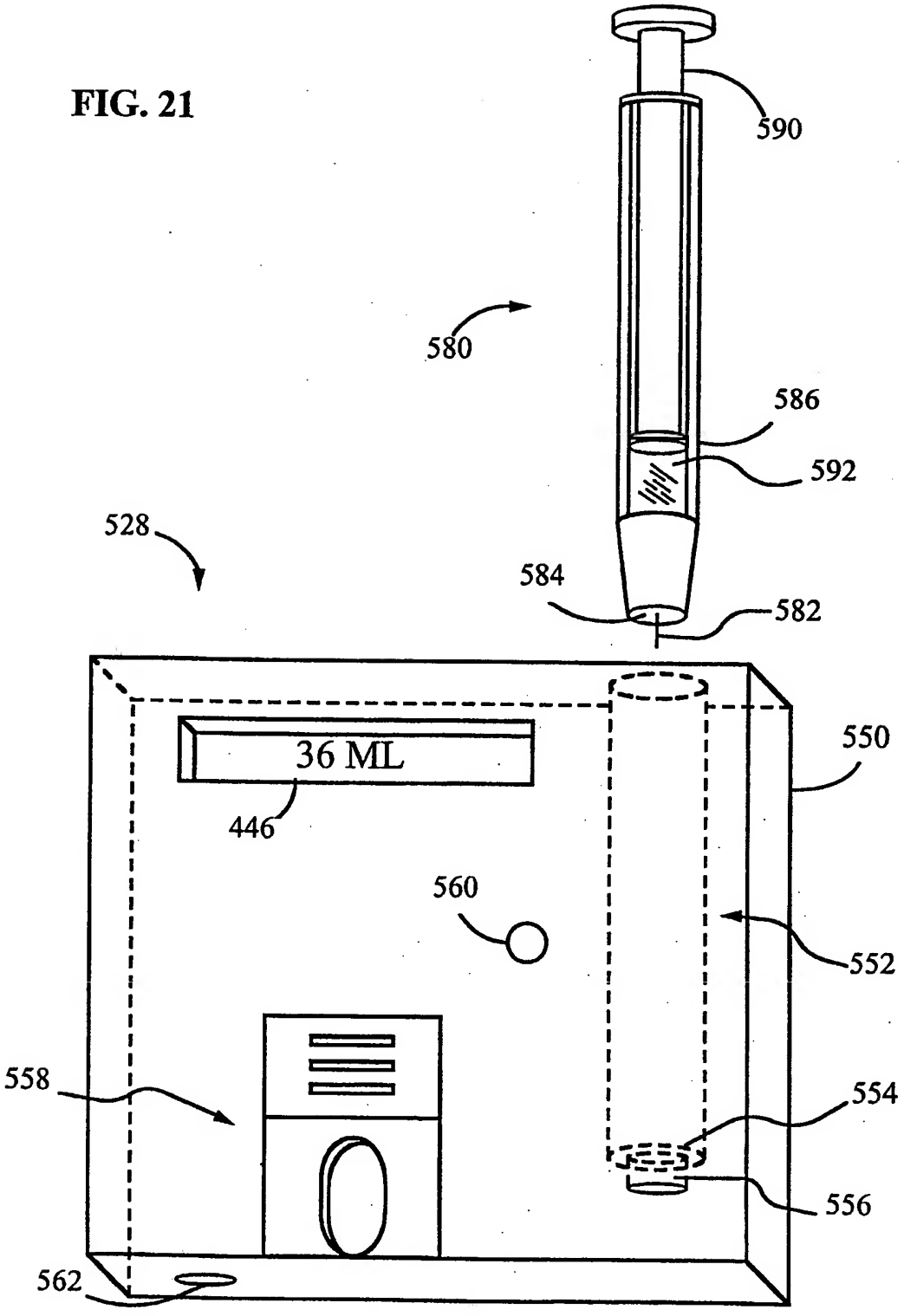


FIG. 22A

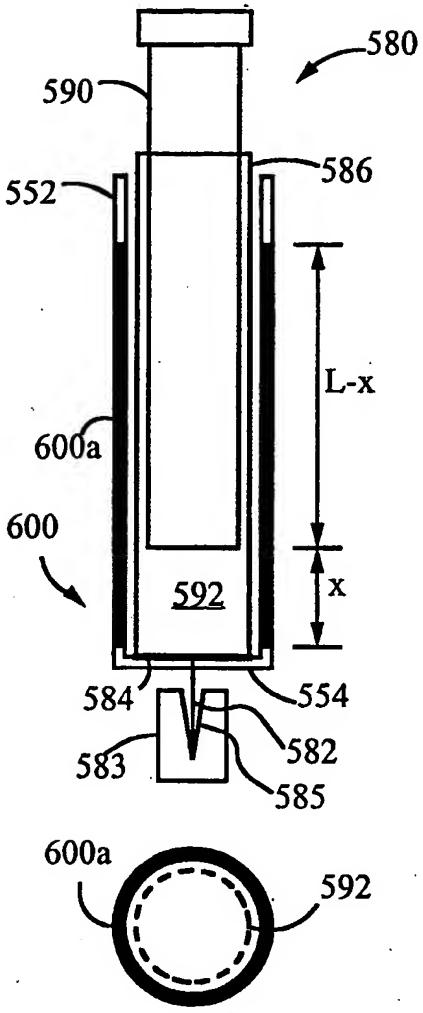
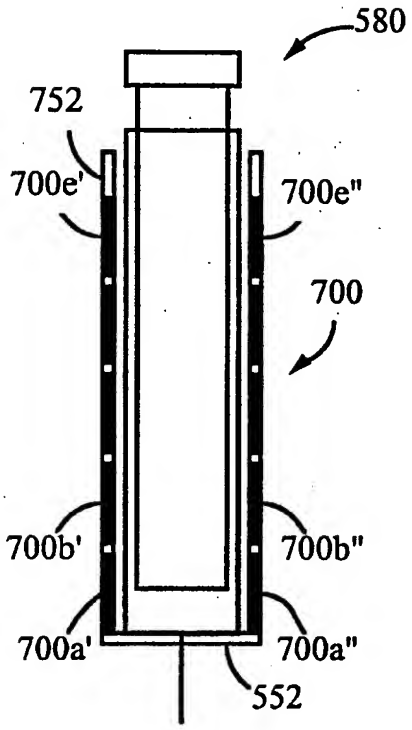


FIG. 22B

FIG. 23A



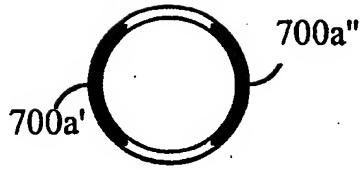


FIG. 23B

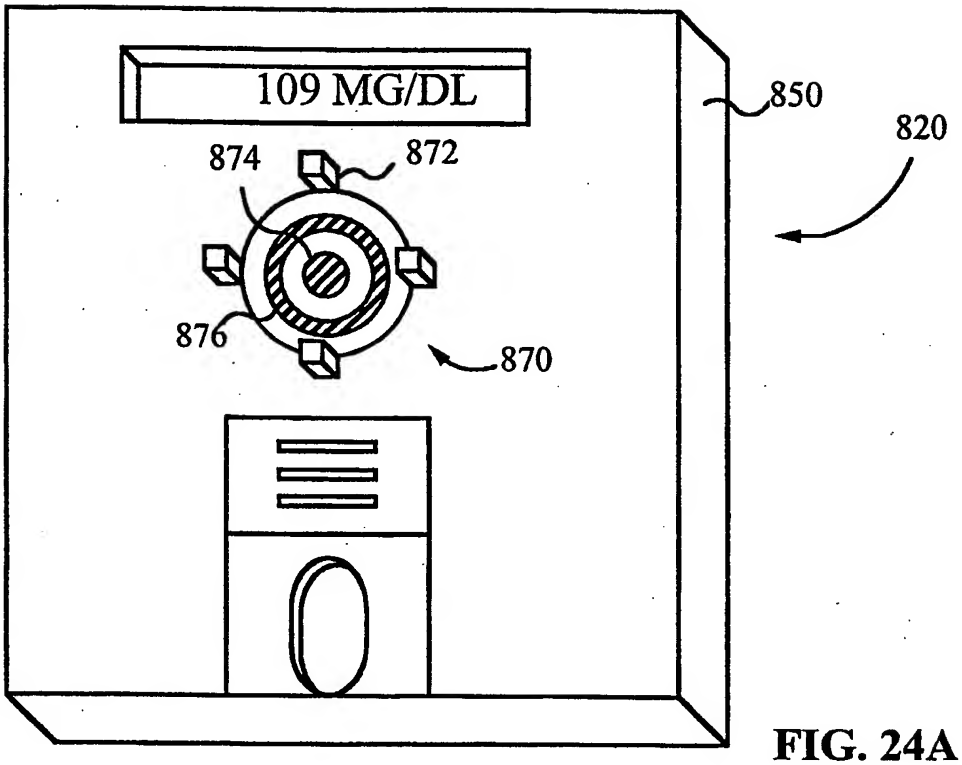


FIG. 24A

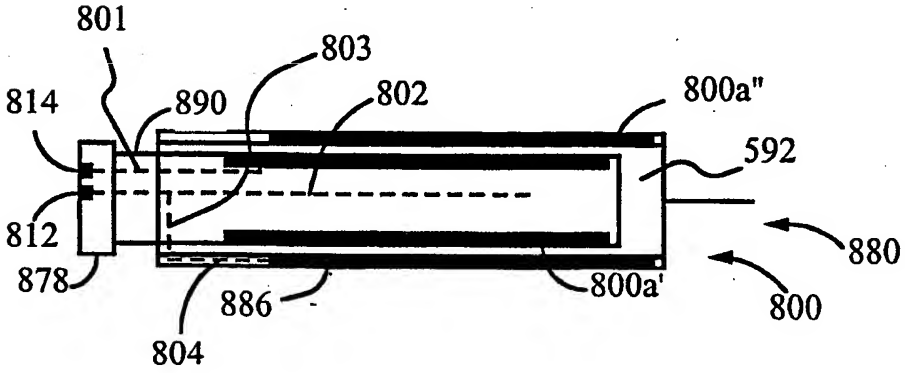


FIG. 24B

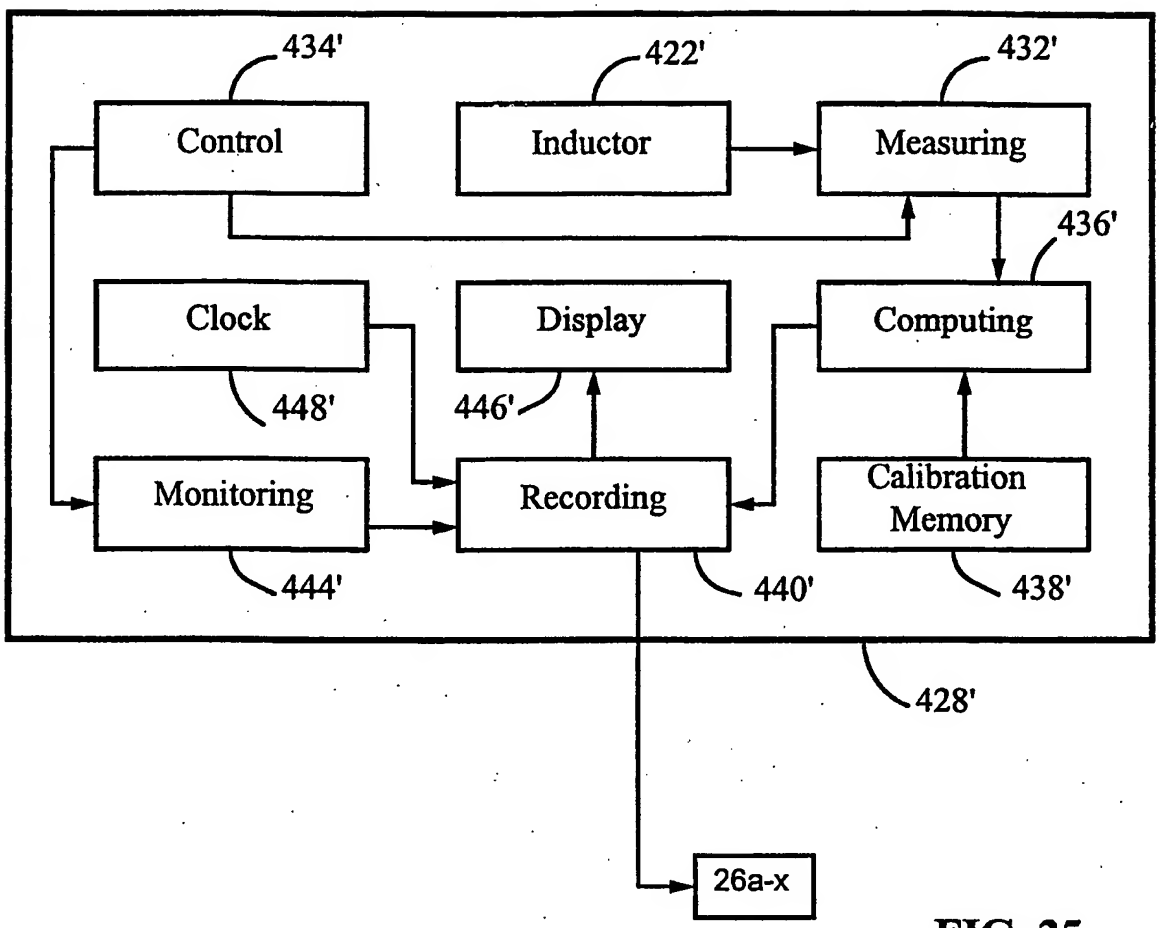
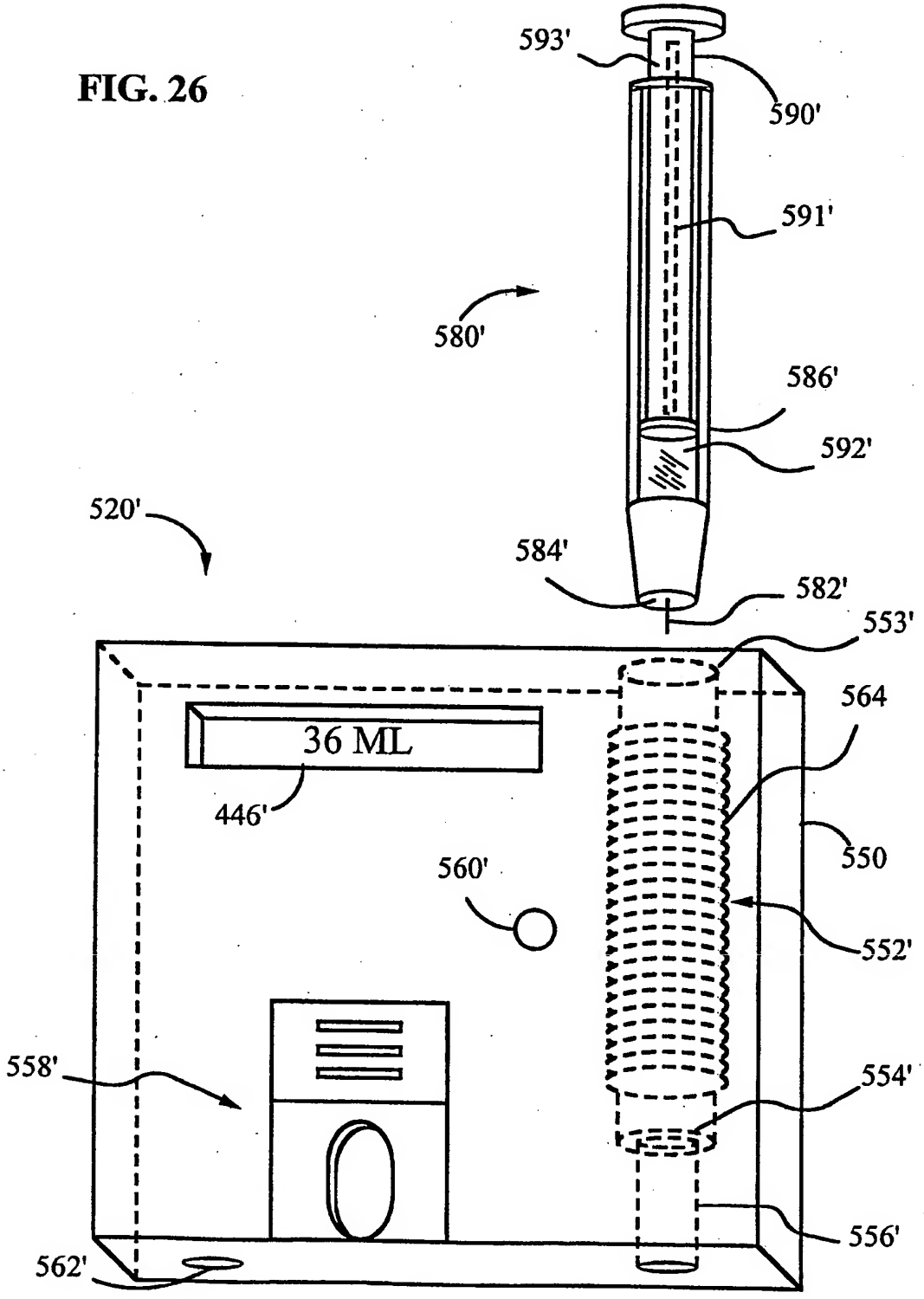

434'
422'
432'
Control
Inductor
Measuring
436'
Clock
Display
Computing
448'
446'
Monitoring
Recording
Calibration Memory
444'
440'
438'
428'
26a-x

FIG. 25

FIG. 26


593'
590'
591'
580'
586'
592'
520'
584'
582'
553'
564
36 ML
446'
550
560'
552'
558'
554'
556'
562'

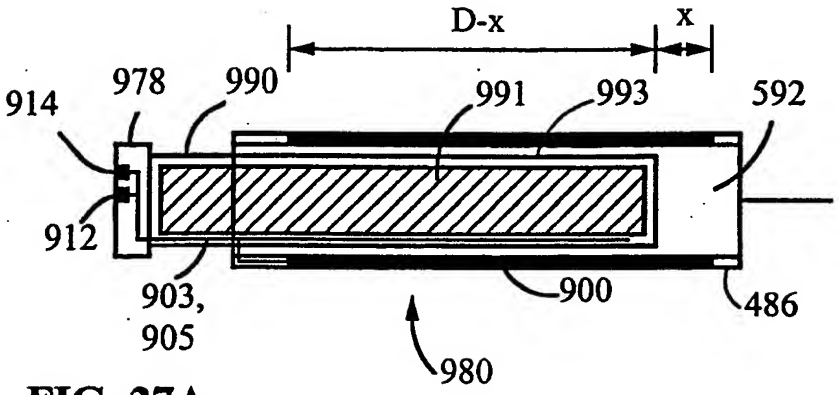


FIG. 27A

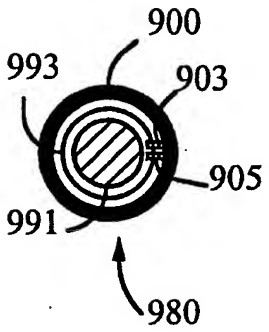


FIG. 27B

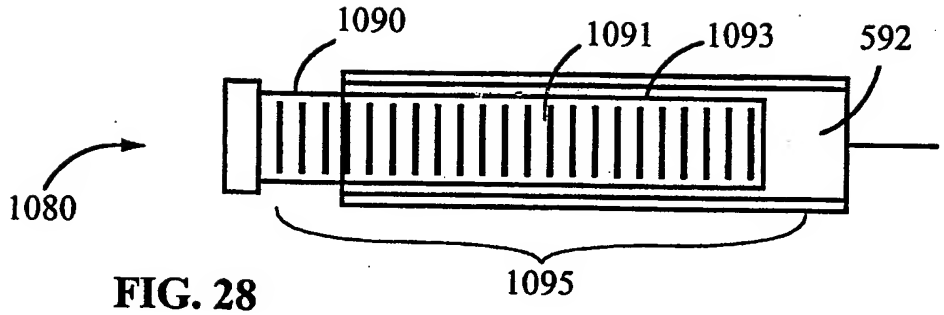


FIG. 28

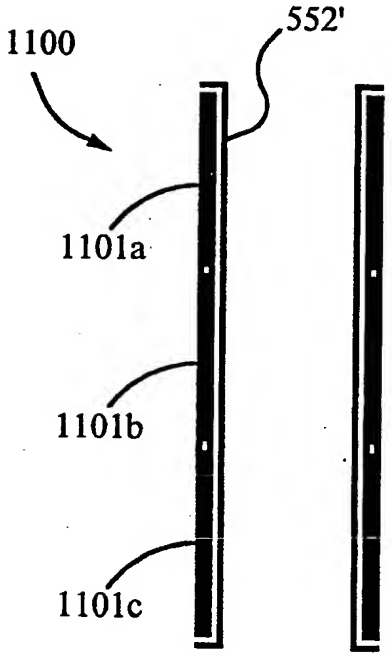


FIG. 29A

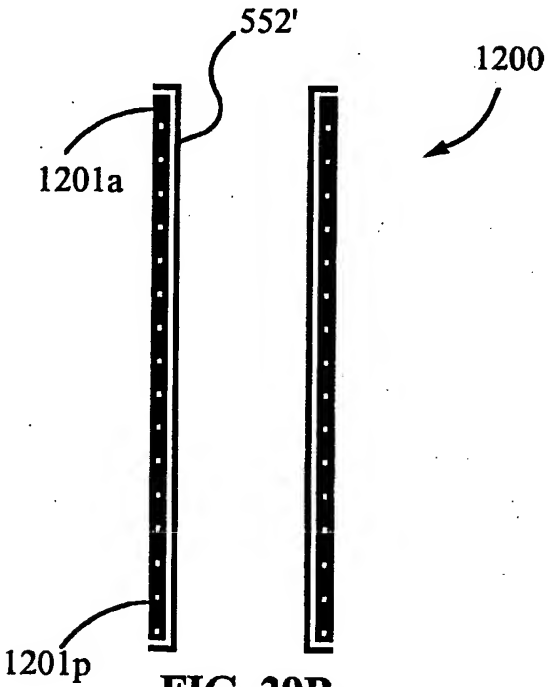


FIG. 29B

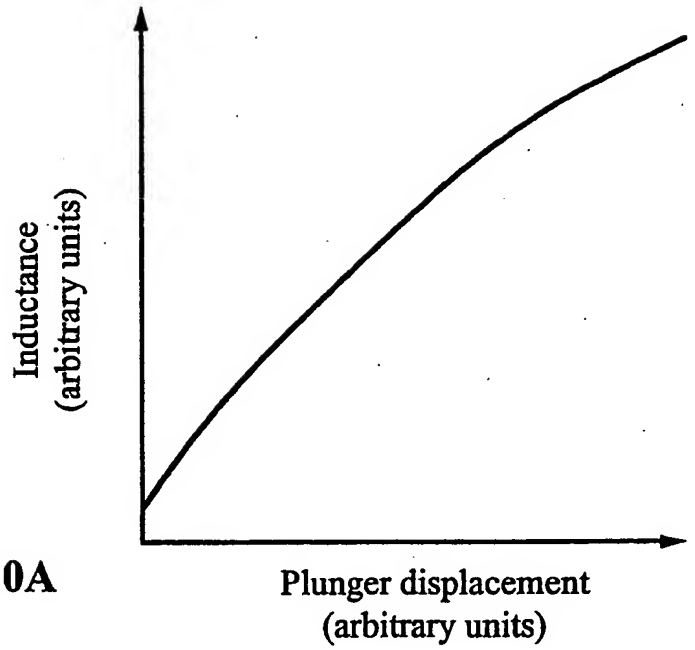


FIG. 30A

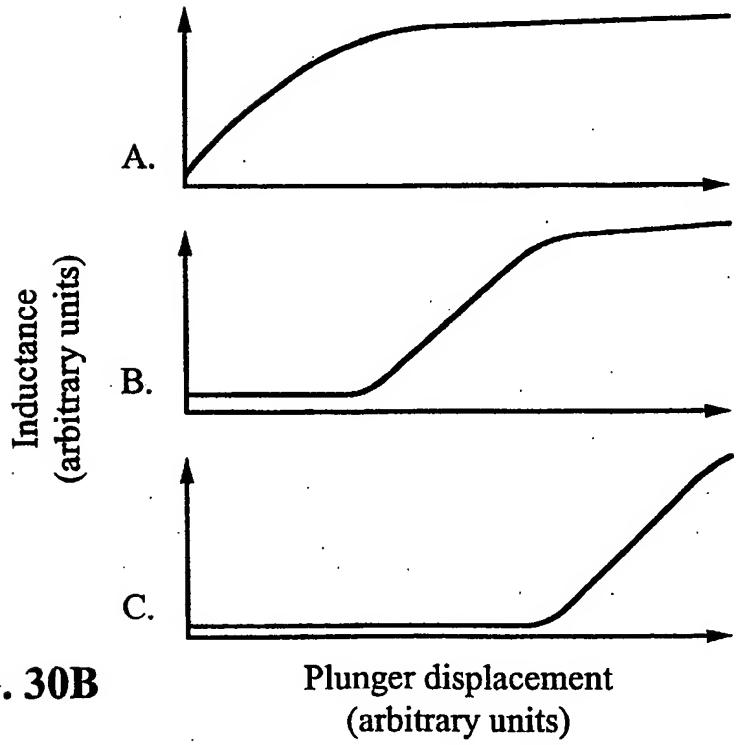


FIG. 30B

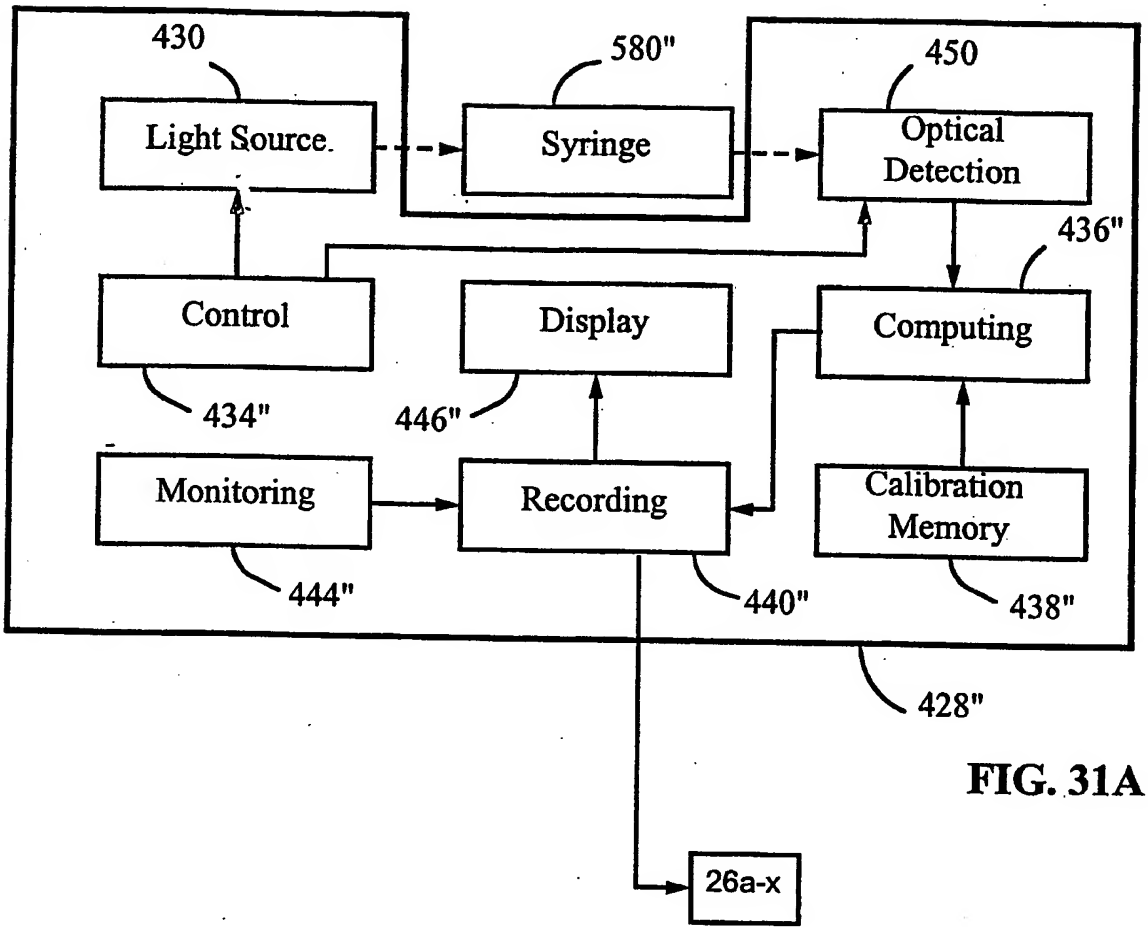


FIG. 31A

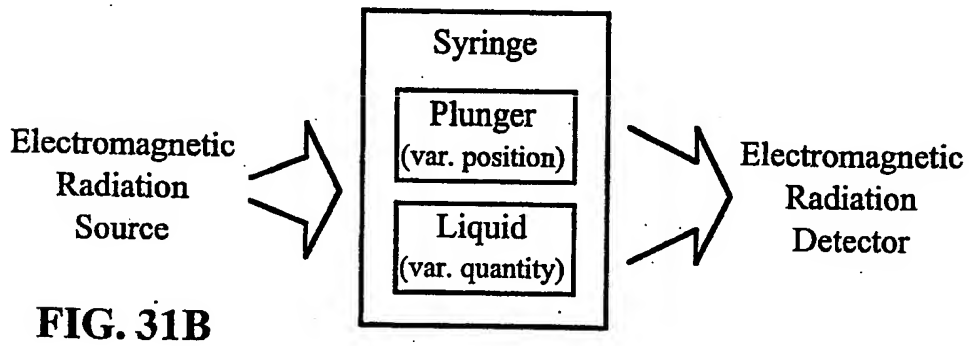
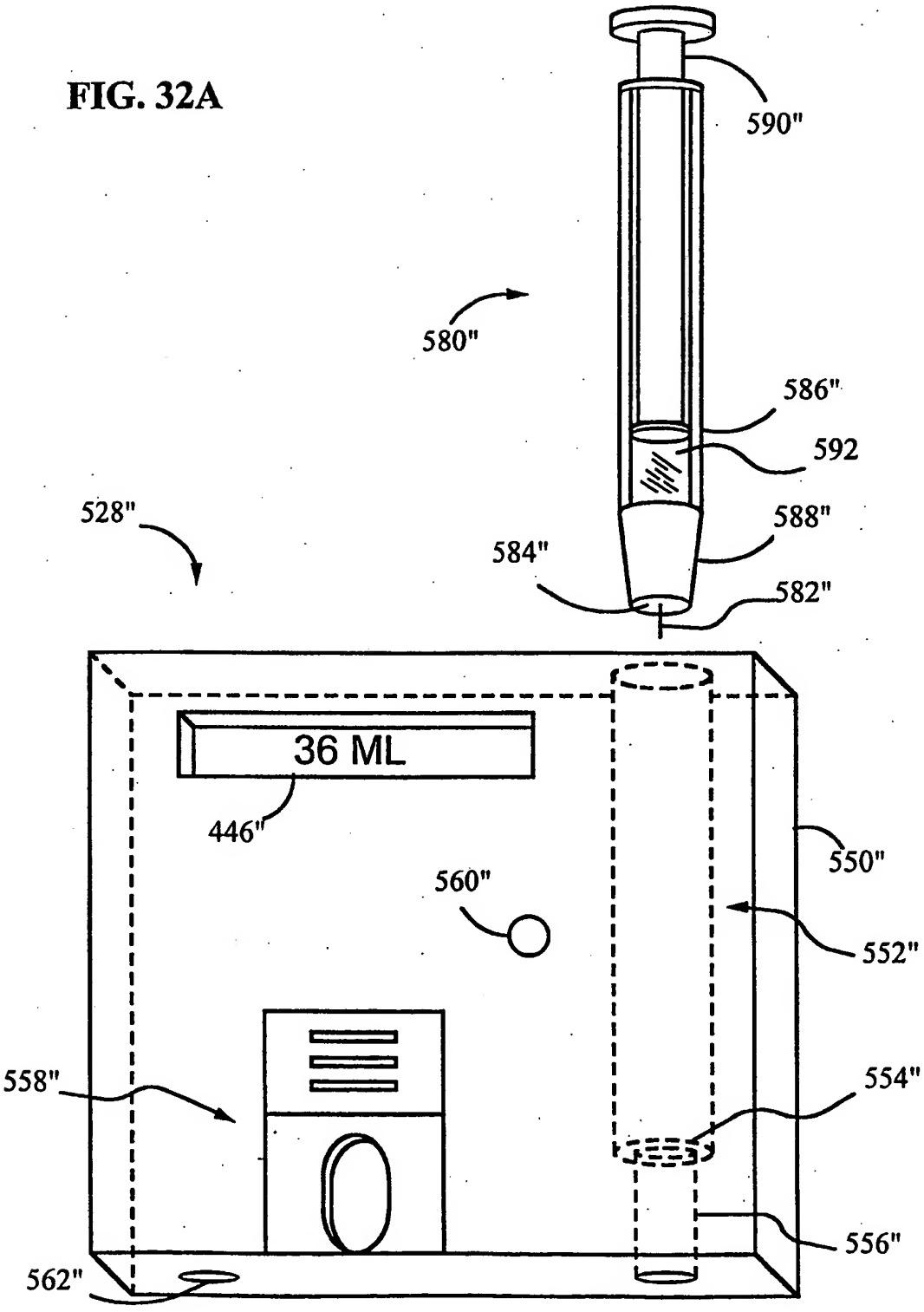


FIG. 31B

FIG. 32A


590"
580"
586"
592
528"
588"
584"
582"
36 ML
446"
550"
560"
552"
558"
554"
556"
562"

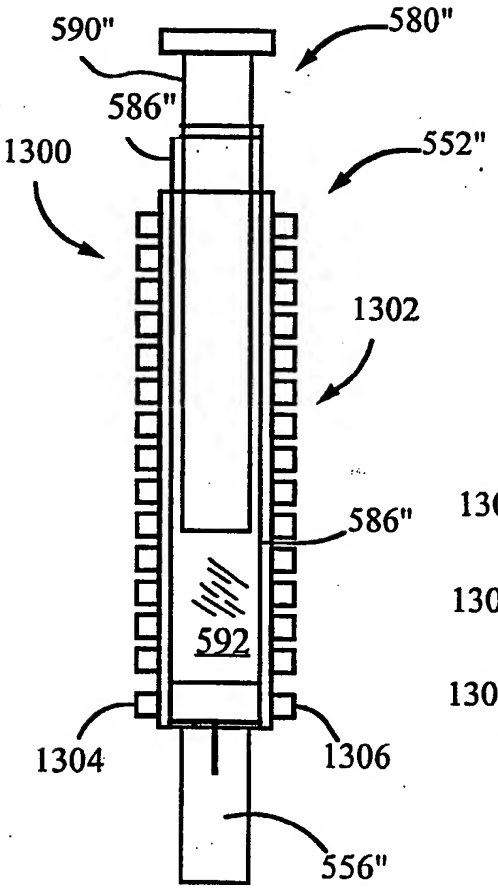


FIG. 32B

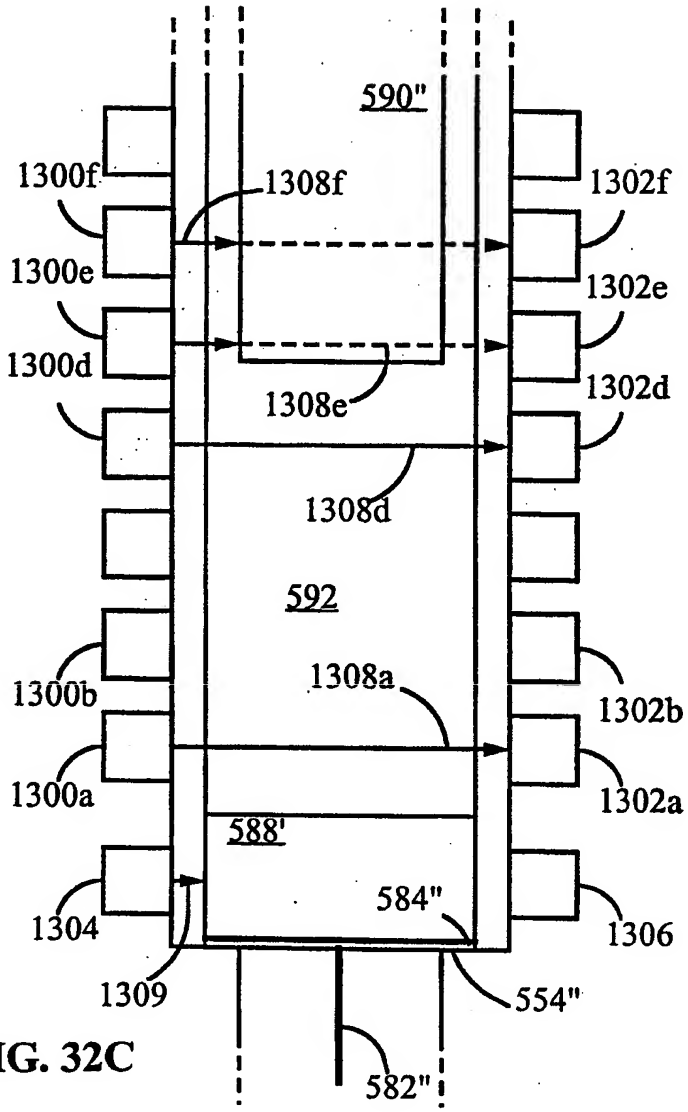


FIG. 32C

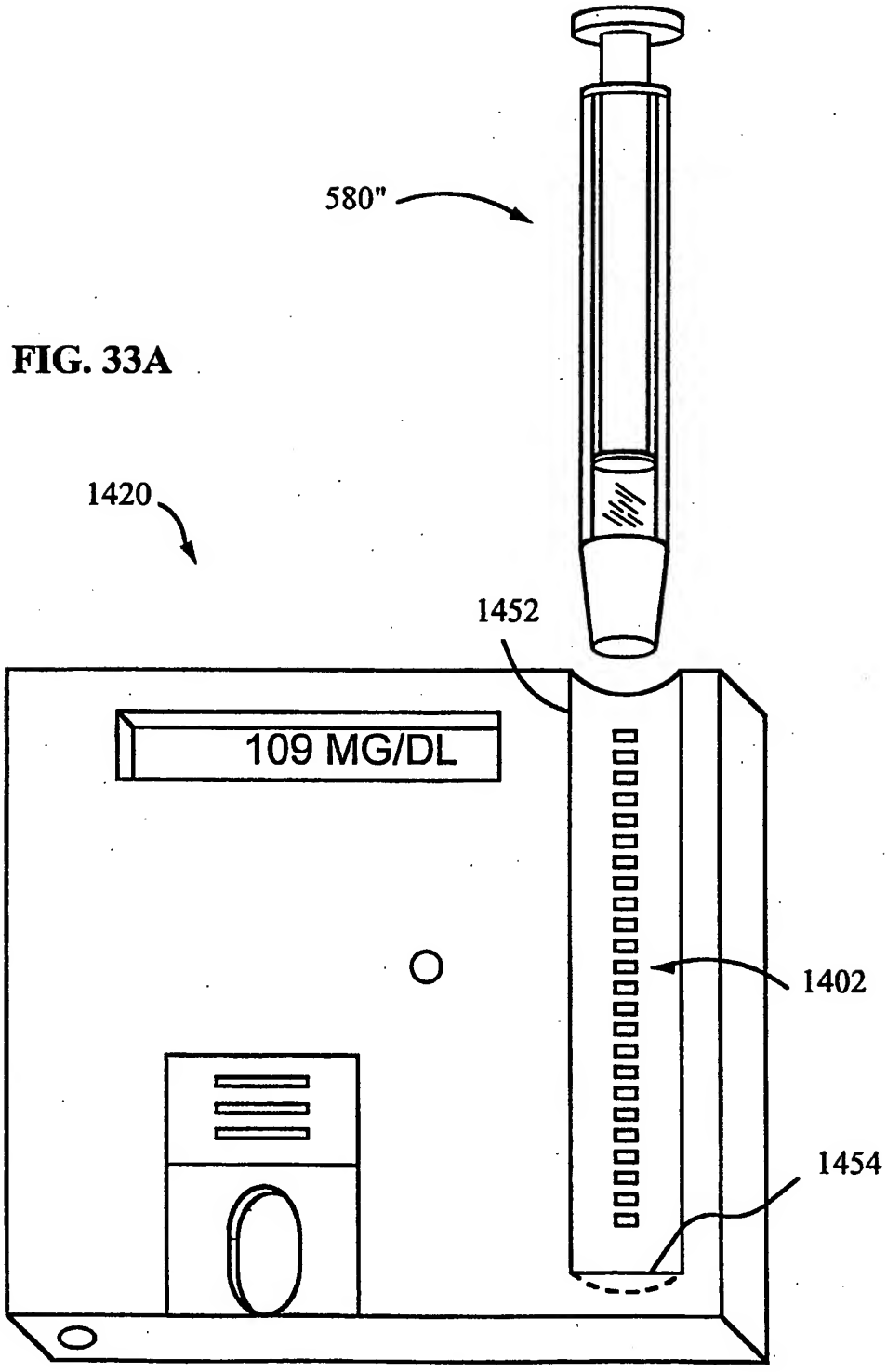


FIG. 33A

FIG. 32D

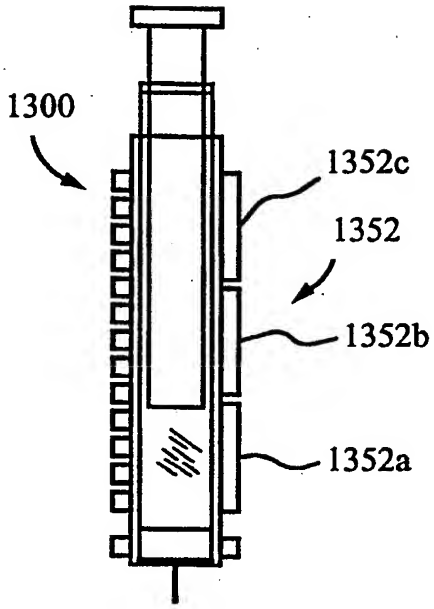


FIG. 32E

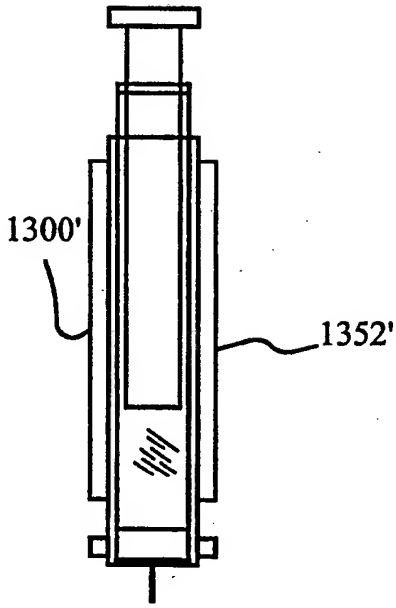


FIG. 33B

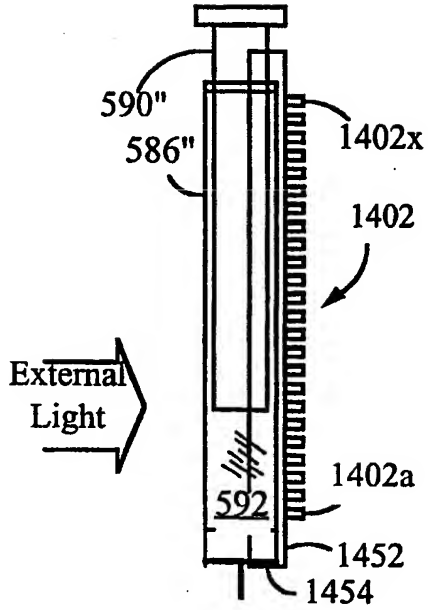
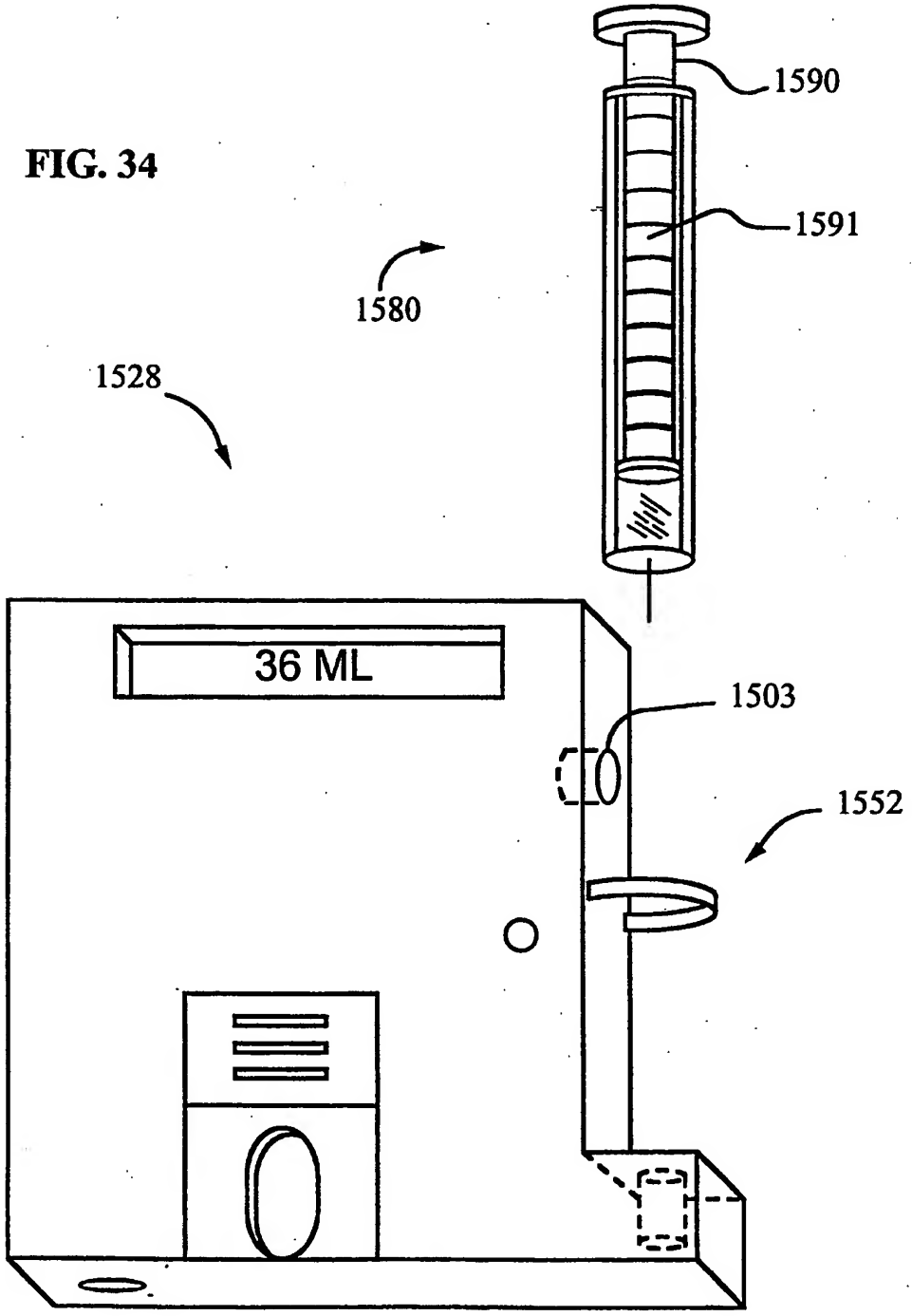


FIG. 34



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A. CLASSIFICATION OF SUBJECT MATTER

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B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST, and WEST 1.2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,711,297 A (ILIFF) 27 January 1998, entire document.	37
Y		1-36, 38-45

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
16 FEBRUARY 2000	17 MAR 2000

Name and mailing address of the ISA/US	Authorized officer
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(54) Title: *IN VIVO* BIOSENSOR APPARATUS AND METHOD OF USE

(57) Abstract

Disclosed are bioluminescent bioreporter integrated circuit devices that detect select analytes in fluids when implanted in the body of an animal. The device comprises a bioreporter that has been genetically engineered to contain a nucleic acid segment that comprises a *cis*–activating response element that is responsive to the selected substance operably linked to a gene encoding a bioluminescent reporter polypeptide. In preferred embodiments, the target analyte is glucose, glucagons, or insulin. Exposure of the bioreporter to the target substance causes the response element to up–regulate the nucleic acid sequence encoding the reporter polypeptide to produce a luminescent response that is detected and quantitated. In illustrative embodiments, the bioreporter device is encapsulated on an integrated circuit that is capable of detecting the emitted light, processing the resultant signal, and then remotely reporting the results. Also disclosed are controlled drug delivery systems capable of being directly or indirectly controlled by the detection device that provide drugs such as insulin to the animal in response to the amount of target analyte present in the body fluids.

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DESCRIPTION

IN VIVO BIOSENSOR APPARATUS AND METHOD OF USE

1.0 BACKGROUND OF THE INVENTION

The present application is a continuing application that claims priority to United States Provisional Application Serial Number 60/110,684, filed December 2, 1998, the entire contents of which is specifically incorporated herein by reference in its entirety.

The United States government has certain rights in the present invention pursuant to grant number R21RR14169-01 from the National Institutes of Health.

1.1 FIELD OF THE INVENTION

The invention generally relates to the field of implantable diagnostic devices (*i.e.* devices deployed within the body of an animal) for monitoring one or more target substances, analytes, or metabolites in the animal. More particularly, the invention provides implantable biosensor devices for monitoring and regulating the level of analytes in the tissues and circulatory system of a human. In illustrative embodiments, the apparatus comprises a biosensor that is utilized to monitor the level of blood glucose in a diabetic or hypoglycemic patient. The disclosed sensors may also be used to control or regulate the delivery of a drug or other pharmaceutical agent from an external or an implantable drug delivery system. For example, the device may form part of an artificial pancreas to regulate insulin dosage in response to the level of glucose detected *in situ*.

1.2 DESCRIPTION OF RELATED ART

1.2.1 BIOSENSORS

Biosensors are hybrid devices combining a biological component with an analytical measuring element. The biological component reacts and/or interacts with the analyte(s) of interest to produce a response measurable by an electronic, optical, or mechanical transducer. The most common configurations presently available utilize immobilized macromolecules such as enzymes or antibodies to form the biological component. Examples of analytes and immobilized macromolecules include: glucose and

immobilized glucose oxidase (*e.g.*, Wilkins *et al.*, 1995); nitrate and immobilized nitrate reductase (Wu *et al.*, 1997); hydrogen peroxide and 2,3-dichlorophenoxyacetic acid and immobilized horseradish peroxidase (Rubtsova *et al.*, 1998); and aspartate and immobilized L-aspartase (Campanella *et al.*, 1995).

1.2.2 WHOLE-CELL BIOSENSORS

A further refinement for biosensors has been developed in recent years that utilizes intact living cells, such as a microorganism, or an eukaryotic cell or cell culture as an alternative to immobilized enzymes. Microbial cells are especially well suited for biosensor technologies; they are physically robust, capable of existing under extremely harsh and widely fluctuating environmental conditions. they possess an extensive repertoire of responses to their environment, and they can be genetically engineered to generate reporter systems that are highly sensitive to these environmental responses. Polynucleotide sequences that comprise specific promoter sequences are operably linked to a gene or a plurality of genes that encode the desired reporter enzyme(s) and then introduced into and maintained within the living cell. When the target analyte is present, the reporter genes are expressed, generating the enzyme(s) responsible for the production of the measured signal. Commonly used reporter systems have utilized either the β-galactosidase (*lacZ*) or catechol-2,3-dioxygenase (*xylE*) enzymes (Kricka, 1993).

Unfortunately, a limitation of these systems has been that following exposure to the target substance(s), the cells must be destructively lysed and the enzyme(s) isolated. This lysis is then followed by the addition of one or more secondary metabolites to yield a colorimetric signal that is proportional to the concentration of enzyme(s) in solution, providing a means to quantify the concentration of the original target substance.

A more recent improvement in such sensors utilizes green fluorescent protein as a reporter system, with the significant advantage that cells do not require destructive assay techniques to produce colorimetric signals. Because a substrate must be added to the green fluorescent protein constructs to first initiate the light response, however, these systems are quite complicated and offer little advantage for detection of analytes *in situ* (Prasher, 1995).

1.2.3 *IN VIVO* SENSORS

The development of an integrated *in vivo* implantable glucose monitor was first reported by Wilkins and Atanasov (1995). This system utilizes glucose oxidase immobilized within a micro-bioreactor. This enzyme catalyzes the oxidation of β-D-glucose by molecular oxygen to yield gluconolactone and hydrogen peroxide, with the concentration of glucose being proportional to the consumption of O_2 or the production of H_2O_2. Unfortunately, the presence of a glucose oxidase inhibitor molecule in the human bloodstream tended to offset proportionality constants, and made the device unsatisfactorily inaccurate for precise glucose monitoring and control (Gough *et al.*, 1997). Also limiting was the device's relatively large size (≈5 × 7 cm), which negated its usefulness as an implantable device.

Although several smaller needle-type and microdialysis glucose sensors have since been developed to circumvent size limitations (Gough *et al.*, 1997, Selam, 1997), their reliance on a glucose oxidase enzyme-based system limits their overall effectiveness and reliability.

Several nonspecific electrochemical sensors have also been investigated as potential *in vivo* glucose sensors (*e.g.*, Yao *et al.*, 1994; Larger *et al.*, 1994), but problems including limited sensitivity, instability, and limited long-term reliability have prevented their wide-spread utilization (Patzer *et al.*, 1995). According to Atanasov *et al.* (1997), continuously functioning implantable glucose biosensors with long-term stability have yet to be achieved.

1.3 DEFICIENCIES IN THE PRIOR ART

Despite a significant miniaturization of biosensors during the past decade, they are still relatively large and obtrusive to serve as ideal implantable devices. Current methodologies using mammalian bioluminescent reporter cells require cell lysis and addition of an exogenous substrate to generate a measurable response. Consequently, these cells cannot serve as continuous on-line monitoring devices.

Therefore, there remains a need for the development of a small implantable monolithic (*i.e.* containing both biological and electrical components constructed on a single substrate layer) bioelectronic monitor that is durable, inexpensive, wireless, and

that can communicate remotely to a drug delivery system to provide the controlled delivery of a therapeutic agent such as insulin.

2.0 SUMMARY OF THE INVENTION

The present invention overcomes these and other inherent limitations in the prior art by providing implantable apparatus and methods for detecting and quantitating particular analytes in the body of an animal. In particular, the invention provides devices for the *in vivo* detection and quantitation of metabolites, drugs, hormones, toxins, or microorganisms such as viruses in a human or animal. In illustrative embodiments, the invention provides a BBIC device useful for the detection of glucose in a human. Such devices provide for the first time an accurate on-line detector for glucose monitoring, and offer the ability to control the administration of pharmaceutical agents *via* an external or implantable drug delivery system. Also disclosed are BBIC devices for detecting the concentration of signature molecules (*i.e.* proteins released from cancer cells, *etc.*), clotting factors, enzymes and the like, and other analytes present in the bloodstream or interstitial fluid. In the area of oncology, the biosensor devices find utility in both initial and remission monitoring, on-line measurement of the effectiveness of chemotherapy, and stimulation/activity of the immune system. Likewise, the biosensor devices are useful in other areas of medicine, including on-line monitoring for enzymes associated with the occurrence of blood clots (strokes, heart attacks, *etc.*), detection and quantitation of clotting factors (maintain level), hormone replacement, continuous drug monitoring (testing for controlled substances in prisoners, military personnel, *etc.*), monitoring of soldiers exposure to sub-lethal exposure to nerve agents and other debilitating agents, monitor levels of compounds affecting mental illness, and the like.

In one embodiment there is provided an implantable monolithic bioelectronic device for detecting an analyte within the body of an animal. In a general sense this device comprises a bioreporter that is operably positioned above a substrate that is on an integrated circuit. The bioreporter is capable of metabolizing the target analyte and emits light consequent to this metabolism when in contact with the analyte. The device further comprises a sensor closely positioned to the integrated circuit that detects the emitted light and generates an electrical signal in proportion to the amount of light generated by the bioreporter. Preferably the entire implantable device is contained within a biocompatible

container that is implanted within the body of the animal in which the analyte detection is desired.

The biocompatible container may be comprised of silicon nitride, silicon oxide, or a suitable polymeric matrix, with exemplary matrices such as polyvinyl alcohol, poly-L-lysine, and alginate being particularly preferred. The polymeric matrix may also further comprise a microporous, mesh-reinforced or a filter-supported hydrogel.

In certain embodiments, it may also be desirable to provide a transparent, biocompatible, bioresistant separator that is operably positioned between the phototransducer and the bioreporter.

The bioreporter preferably comprises a plurality of eukaryotic or prokaryotic cells that produce a bioluminescent reporter polypeptide in response to the presence of the target analyte. Prokaryotic cells such as one or more strains of bacteria, and eukaryotic cells such as mammalian cells are particularly preferred. Exemplary mammalian cells are human cells such as islet β-cells, immortal stem cells, or hepatic cells, with immortal stem cells being particularly preferred.

These cells preferably comprise one or more nucleic acid segments that encode a luciferase polypeptide or a green fluorescent protein that is produced by the cells in response to the presence of the analyte. Preferably the nucleic acid segment encodes an *Aqueorea Victoria, Renilla reniformis,* or a humanized green fluorescent protein, or more preferably, a bacterial Lux polypeptide, such as the LuxA, LuxB, LuxC, LuxD, or LuxE polypeptide, or the LuxAB or LuxCDE fused polypeptides described herein.

Exemplary bacterial *lux* gene sequences that may be employed to prepare the genetic constructs include the *Vibrio fischerii* or more preferably, the *Xenorhabdus luminescens luxA, luxB, luxC, luxD, luxE, luxAB,* or *luxCDE* genes.

Exemplary *lux* gene sequences that may be employed for preparation of the genetic constructs as described herein include the gene sequences disclosed in SEQ ID NO:1. Exemplary Lux polypeptide sequences are disclosed in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6.

The Lux polypeptides preferably comprise at least a 10 contiguous amino acid sequence from one or more of the polypeptide sequences disclosed in SEQ ID NO:2 through SEQ ID NO:6. More preferably the Lux polypeptides comprise at least a 15 contiguous amino acid sequence from one or more of the polypeptide sequences disclosed

in SEQ ID NO:2 through SEQ ID NO:6, and more preferably still, comprise at least a 20 contiguous amino acid sequence from one or more of the polypeptide sequences disclosed in SEQ ID NO:2 through SEQ ID NO:6.

Such polypeptides are preferably encoded by a nucleic acid sequence that comprises at least 20, at least 25, at least 30, at least 35, at least 40, or at least 45 or more contiguous nucleotides from SEQ ID NO:1.

The expression of the Lux-encoding nucleic acid segments is preferably regulated by a nucleic acid regulatory sequence operably linked to the Lux-encoding segment. Preferably this regulatory sequence comprises a *cis*-acting element that is responsive to the presence of the target analyte. Exemplary *cis*-acting response elements are selected from the group consisting of an S14 gene sequence, a hepatic L-pyruvate kinase gene sequence, a hepatic 6-phosphofructo-2-kinase gene sequence, a β-islets insulin gene sequence, a mesangial transforming growth factor-β gene sequence, and an acetyl-coenzyme-A carboxylase gene sequence.

In an illustrative embodiment, the *cis*-acting response element comprises a contiguous nucleotide sequence from a β-islets insulin gene sequence or a hepatic L-pyruvate kinase gene sequence. Expression of the nucleic acid sequence is preferably regulated by a promoter sequence such as the one derived from an L-pyruvate kinase-encoding gene described herein.

The device may further comprise a wireless transmitter, an antenna, and a source of nutrients capable of sustaining the bioreporter cells. Likewise the biocompatible container enclosing the bioreporter may further comprise a membrane that is permeable to the analyte but not to the bioreporter cells themselves. Such a semi-permeable membrane permits analytes to flow freely from the bodily fluid into the detector device, but restricts the migration of bioreporter cells from the device into the surrounding tissues or circulatory system of the body in which the device is implanted.

In one embodiment, the integrated circuit is a complementary metal oxide semiconductor (CMOS) integrated circuit. The integrated circuit may comprise one or more phototransducer, that themselves may be comprised of one or more photodiodes. Likewise, the integrated circuit may also further comprise a photodiode, a current-to-frequency converter, a digital counter, and/or a transmitter that is capable of transmitting either digital or analog data.

The invention also provides an implantable controlled drug delivery system that comprises both the BBIC device and an implantable drug delivery pump that is capable of being operably controlled by the BBIC and that is capable of delivering the drug to the body of the animal in response to controls by the device. The invention also concerns a method of providing a controlled supply of a drug to a patient in need thereof. The method generally involves implanting within the body of the patient the controlled drug delivery system

The invention also provides a method of determining the amount of a drug required by a patient in need thereof, such as in the case of giving a diabetic patient an appropriate amount of insulin. The method generally involves implanting within the body of the diabetic patient one or more BBIC devices that are responsive to either glucose, glucagons, insulin, or another glucose metabolite, and determining the amount of insulin required by the patient based upon the levels of the analyte detected in the body fluids by the device. When the device indicates that higher levels of insulin are required, the appropriate control signal can be sent to the drug delivery system and more insulin is injected into the body. When the device indicates that lower levels of insulin are required, then the appropriate control signal can be sent to the drug delivery system and less insulin can be administered. Such "real-time" monitoring of glucose in the body of the animal permits for controlled release of insulin throughout the day, and obviates the need for daily or more frequent injections of insulin that may either be too much or too little for the particular time of administration. This affords a more cost-effective administration of the drug, and also provides a more stable dosing of the insulin to the patient on an "as needed" basis.

The invention also provides a kit for the detection of an analyte, and such kits generally will include one or more of the disclosed BBIC devices in combination with appropriate instructions for using the detection device. Such kits may also routinely contain one or more standardized reference solutions for calibrating the device, and may also include suitable storage or nutrient medium for sustaining the bioreporter cells either during storage or during use once implanted within the body of the animal. In the case of therapeutic kits, such kits will also generally include one or more controlled delivery systems for administration of the drug to the body of the animal.

The invention also provides a method of regulating the blood glucose level of an animal in need thereof. This method generally comprises monitoring the level of glucose in the bloodstream or inerstitial fluid of the patient using the BBIC device, and administering to the patient an effective amount of an insulin composition sufficient to regulate the blood glucose level.

This new type of bioluminescence-based bioreporter is capable of monitoring target substances without the disadvantageous requirement that cells be destroyed to produce the measurable signal. This allows for monitoring to occur continuously, on-line and in real-time (Simpson *et al.*, 1998a, 1998b). These cells rely on luciferase genes (designated *lux* in prokaryotes and *luc* in eukaryotes) for the reporter enzyme system. U. S. Patent Appl. Ser. No. 08/978,439 and Intl. Pat. Appl. Ser. No. PCT/US98/25295 (each of which is specifically incorporated herein by reference in its entirety) disclose a self-contained miniature bioluminescence bioreporter integrated circuit ("BBIC") that was designed to detect specific molecular targets *ex situ* or *ex vivo*.

The present invention concerns an implantable, or an *in situ* or an *in vivo* BBIC device that is capable of being implanted within the body of an animal, and that is capable of detecting the concentration of one or more analytes present within the animal. The implantable monolithic bioelectronic device of the present invention generally comprises a substrate, a bioreporter capable of responding to a particular substance by the emission of light, a container affixed to the substrate capable of holding the bioreporter, an integrated circuit on the substrate including a phototransducer operative to generate an electrical signal in response to the light wherein the signal indicates the concentration of the substance; and a biocompatible housing that is capable of being implanted within the body of an animal, with that portion of the housing covering the bioreporter container comprising a semi-permeable membrane that permits passage of the analyte from the body of the animal to contact the biosensor, but restricts the bioreporter molecules from diffusing into the body of the animal that contains the implanted device. The bioreporter may be in solution, that is a cell suspension, and entrapped in the container by the semi-permeable membrane, or alternatively the bioreporter may be encapsulated in a selectively permeable polymer matrix that is capable of allowing the selected substance in solution reach the bioreporter. Preferably, the matrix is optically clear.

The apparatus may further comprise a layer of bioresistant/biocompatible material between the substrate and the container, such a layer of silicon nitride. The integrated circuit is preferably a CMOS integrated circuit, and the phototransducer is preferably a photodiode.

The integrated circuit may also include a current to frequency converter and/or a digital counter. Additionally, the integrated circuit may also include one or more transmitters. Such transmitters may be wireless, or conventionally wired. In preferred embodiment, the apparatus also includes a drug delivery device capable of receiving transmissions from the transmitter.

A further embodiment of the invention is an implantable apparatus for detecting a selected substance in solution, which comprises an integrated circuit including a phototransducer adapted to input an electrical signal into the circuit in response to light, a bioreporter capable of responding to selected substance in solution by emitting light, the reporter adapted to contact the substance; and a transparent, biocompatible, and bioresistant separator positioned between the phototransducer and the bioreporter to enable light emitted from the bioreporter to strike the phototransducer. In a preferred embodiment of the present invention, the selected substance is glucose. The bioreporter may be a mammalian cell that contains a nucleotide sequence that encodes one or more luminescent reporter molecules. Such a nucelotide sequence may comprise one or more *lux* genes. In a preferred embodiment the *lux* genes comprise both *luxCDE* genes and fused *luxAB* genes. In one embodiment, these *lux* genes are derived from *Xenorhabdus luminescens*. The *lux* genes may be regulated by a nucleic acid sequence comprising one or more *cis*-acting glucose response elements. In an illustrative embodiment, the glucose response element may be derived from the β-islets or hepatic L-pyruvate kinase gene. In a highly preferred embodiment the p.LPK.Luc_{FF} plasmid is used to provide one or more glucose response elements and the L-pyruvate kinase promoter to drive the expression of one or more *lux* genes. The cells constituting the bioreporter may be in suspension, entrapped in place on the IC by a semi-permeable membrane. Alternatively the cells constituting the bioreporter may be encapsulated in a polymer matrix affixed to IC. Such a matrix may be permeable to the selected substance in solution.

A further embodiment of the invention concerns an implantable monolithic bioelectronic device for detecting a selected substance in body fluid. This device

generally comprises a biocompatible housing; a bioreporter capable of responding to a selected substance by emitting; and, a sensor capable of generating an electrical signal in response to the reception of the emitted light. Such a device may also include a transparent, bioresistant and biocompatible separator positioned between the bioreporter and the sensor and a semi-permeable membrane positioned in the biocompatible housing so that the selected substance can access the bioreporter.

A standard integrated circuit (IC) is coated with a layer of insulating material such as silicon dioxide or silicon nitride. This process is called passivation and serves to protect the surface of the chip from moisture, contamination, and mechanical damage. BBICs require a second coating that must be biocompatible and bioresistant, must protect the OASIC from chemical stresses, must be optically tuned to efficiently transmit the light from the material under test, must adhere to an oxide coating, must be pin-hole free, and must be able to be patterned in order to form openings over the bonding pads and whatever structures that might be needed to maintain the bioreporter or collect a sample.

The present invention contemplates that the components of the biosensor may be packaged in kit form. Kits may comprise, in suitable container means, one or more bioreporters and an integrated circuit including a phototransducer. Kits may further comprise a drug delivery device.

3.0 Brief Description of the Drawings

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein. Illustrative embodiments of the present invention are depicted in the drawings, with like numerals being used to refer to like and corresponding parts of the various drawings.

FIG. 1 shows a perspective view of one illustrative embodiment of the invention.

FIG. 2 shows a side view of an illustrative embodiment of the present invention.

FIG. 3 shows a block diagram of an illustrative embodiment of the integrated circuit.

FIG. 4A shows a high-quality photodetector that can be made using a standard N-well CMOS process.

FIG. 4B shows two photodetector structures fabricated in a silicon-on-insulator CMOS process: on the left, a lateral PIN detector; on the right, a device similar to left except that the junction is formed with a Schottky junction.

FIG. 5A shows a simple photodiode consisting of a P-diffusion layer, an N-well, and a P-substrate.

FIG. 5B shows a circuit using a large area photodiode for efficient light collection, and a small-area diode in a feedback loop to supply the forward bias current that cancels out the photocurrent.

FIG. 5C shows a circuit using correlated double sampling (CDS) to minimize the effects of low frequency (flicker) amplifier noise as well as time or temperature dependent variations in the amplifier offset voltage.

FIG. 6 shows the current-to-frequency converter architecture of the apparatus.

FIG. 7 shows a prototype BBIC biosensor.

FIG. 8 shows a minimum detectable concentration of toluene as a function of integration time for the prototype BBIC employing the bioreporter *Pseudomonas putida* TVA8.

FIG. 9 shows the schematic representation of a peritoneal glucose biosensor and insulin pump.

FIG. 10A shows a schematic representation of an implantable biosensor containing two separate photodetectors with the bioreporters responding to either an increase or decrease in glucose concentrations.

FIG. 10B shows a side view of biosensor showing silastic covering.

FIG. 10C shows a schematic representaion of the utilization of a selectably permeable membrane to protect bioreporters from the immune response.

4.0 DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The luciferase system has been adapted for use in biosensors *in vivo*. In prokaryotes, the *lux* system consists of a luciferase composed of two subunits coded for by the genes *luxA* and *luxB* that oxidize a long chain fatty aldehyde to the corresponding fatty acid resulting in a blue-green light emission at an approximate wavelength of 490 nm (Tu and Mager, 1995). The system also contains a multienzyme fatty acid reductase consisting of three proteins, a reductase encoded by *luxC*, a transferase encoded by *luxD*,

and a synthetase encoded by *luxE* that convert and recycle the fatty acid to the aldehyde substrate. The genes are contained on a single operon, allowing for the cloning of the complete *lux* gene cassette downstream from user-specific promoters for the utilization of bioluminescence to monitor gene expression. The majority of bioluminescent bioreporters consist of Gram-negative organisms engineered to detect and monitor critically important chemical and environmental stressors (Ramanathan *et al.*, 1997, Steinberg *et al.*, 1995). Luciferase fusions in Gram-positive bacteria, as well as in yeast cell lines, are also being successfully performed (Andrew and Roberts, 1993, Srikantha *et al.*, 1996).

Eukaryotic luciferase genes cloned into bacterial reporters include the firefly luciferase (*luc*) producing light near 560 nm and the click beetle luciferase (*lucOR*) emitting light near 595 nm (Cebolla *et al.*, 1995, Hastings, 1996). Eukaryotic bioreporters have been designed to monitor glucose concentrations in rat islet β-cells (Kennedy *et al.*, 1997), steroid activity in HeLa cells (Gagne *et al.*, 1994), ultraviolet light effects in mouse fibroblast cells (Filatov *et al.*, 1996), toxicity effects in human liver cancer cells (Anderson *et al.*, 1995), estrogenic and antiestrogenic compounds in breast cancer cell lines (Demirpence *et al.*, 1995), and erythropoiten gene induction in human hepatoma cell lines (Gupta and Goldwasser, 1996). To date, most eukaryotic bioluminescent reporters require cell destruction and the addition of an exogenous substrate, usually luciferin, to generate a measurable luminescent response.

Green fluorescent protein ("GFP") is also routinely used as a reporter system, with the significant advantage that cells do not require destructive assay techniques to produce colorimetric signals (Hanakam *et al.*, 1996; Grygorczyk *et al.*, 1996; Siegel and Isacoff, 1997; Biondi *et al.*, 1998). However, a substrate must be added to the GFP constructs to first initiate the light response (Prasher, 1995). Humanized GFP cDNA has been developed which is specifically adapted for high-level expression in mammalian cells, especially those of human origin (Zolotukhin 1996). Humanized GFP can be efficiently inserted into mammalian cells using viral vectors (Levy *et al.*, 1996; Gram *et al.*, 1998).

Detection of the bioluminescent signal from the reporter organisms is achieved through the use of optical transducers, including photomultiplier tubes, photodiodes, microchannel plates, photographic films, and charge-coupled devices. Light is collected and transferred to the transducer through lenses, fiber optic cables, or liquid light guides.

However, applications requiring small volumes, remote detection, or multiple parallel sensing necessitate a new type of instrumentation that is small and portable, yet maintains a high degree of sensitivity.

4.1 Overview of the System

The present invention describes an implantable BBIC that detects selected substances. The bioreporter is a genetically engineered cell line in which the nucleic acid sequence contains a *cis*-activating response element that is responsive to the selected substance. In preferred embodiments, the selected substance is glucose. Exposure of the bioreporter to the selected substances causes the response element to up-regulate a nucleic acid sequence that encodes one or more polypeptides that generate a luminescent response. In a preferred embodiment, the luminescent response is generated by a prokaryotic *lux* system.

The function of the IC portion of the BBIC is to detect, filter, amplify, digitize, and report the bioluminescent signal. In effect, the IC serves as a complete laboratory instrument-on-a-chip: a microluminometer.

Silicon-based ICs can detect optical signals in the near ultraviolet, visible, and near infrared regions using the PN junctions normally used to form transistors (Simpson *et al.*, 1999a). Using an n-well/p-substrate photodiode in a 0.5-μm bulk CMOS IC process, an ~66% quantum efficiency has been measured at the 490-nm bioluminescent wavelength (Simpson *et al.*, 1999b). A variety of signal-processing schemes can be employed. However, counting the pulses from a current-to-frequency converter circuit forms a long time-constant integrator and is the causal portion of the matched filter for a low-level bioluminescent signal in white noise. Using the photodiode mentioned above with this signal-processing scheme, an rms noise level of 175 electrons/second was measured for a 13-minute integration time, corresponding to a detection limit of ~500 photons/second (Simpson *et al.*, 1999b).

A prototype BBIC was constructed by placing the toluene sensitive bioreporter, *P. putida* TVA8, above a custom integrated microluminometer. FIG. 6 shows the prototype BBIC (including the bioreporter enclosure) as used in the characterization studies (Simpson *et al.*, 1998b: Simpson *et al.*, 1998c; Simpson *et al.*, 1998d).

With no luminescent signal coming from the cells, multiple measurements were taken with the integration time set to 1-minute. Leakage currents produced a signal of ~6 counts/minute with a standard deviation (σ) of 0.22 counts/minute. As expected, the σ decreased with the square root of the integration time. Longer integration times were produced off-line by summing 1-minute measurements.

Bioluminescence was induced in the BBIC cells and a control sample of cells by exposure to toluene vapor. From the control sample measurements, we estimate that the toluene concentration was no more than 1 ppm. A signal of 12 counts/minute (6 counts/minute above background) was measured. From previous measurements, *P. putida* TVA8 is known to have a linear response to toluene concentration until saturating when the concentration reaches a level of approximately 10 ppm. The minimum detectable toluene concentration for this BBIC as a function of integration time is shown in FIG. 8. In general, the minimum detectable concentration is also a function of the number of bioreporter cells and the area of the photodiode.

A naphthalene-sensitive BBIC was produced using the microluminometer described above and the bioreporter *P. fluorescens* 5RL. Using the same experimental procedure described above, this BBIC was exposed to naphthalene vapor with a concentration of approximately 10 ppm. A signal of 240 counts/minute was recorded.

To eliminate the need for the addition of exogenous substrate, cells must themselves supply the appropriate substrate for the luciferase. In the bacterial system the substrate is generated by a fatty acid reductase complex coded for by the *luxCDE* genes. This enzyme complex reduces short chain fatty acids to the corresponding aldehyde. The luciferase then oxidizes the aldehyde to the corresponding fatty acid. The preferred fatty acid for this reaction is myristic acid, which is present in eukaryotic organisms (Rudnick *et al.*, 1993). Myristic acid is usually involved in the myristoylation of the amino terminus that is associated with membrane attachment (Borgese *et al.*, 1996, Brand *et al.*, 1996).

In a preferred embodiment, the bioreporter for glucose monitoring will be a mammalian bioluminescent reporter cell line that has been genetically engineered to express luminescence in response to glucose concentrations on a continuous basis, without the need for cell destruction and exogenous substrate addition. Current methodologies using mammalian bioluminescent reporter cells require cell lysis and addition of an

exogenous substrate to generate a measurable response. Consequently, these cells cannot serve as continuous on-line monitoring devices. In a preferred embodiment, this new cell line is constructed with a bioluminescent reporter utilizing the *luxAB* and *luxCDE* genes from *X. luminescens* incorporated into a plasmid-based system designated p.LPK.Luc_{FF} which contains a eukaryotic *luc* gene able to respond to glucose concentrations. Replacement of the *luc* gene with the *luxAB* gene will allow for bioluminescence measurements to occur in real-time with glucose concentrations, negating the requirement for cell destruction and substrate addition.

To form an implantable, glucose-monitoring BBIC, the bioreporters may be entrapped in a container behind a semi-permeable membrane that keeps them in place over the IC photodetector. Alternatively the bioreporter may be encased in a polymer matrix. The BBIC is enclosed in a biocompatible housing with a semi-permeable membrane covering the bioreporter region. This membrane allows glucose to pass to the bioreporters, yet stops the passage of larger molecules that could interfere with the glucose measurement. When the glucose reaches the bioreporter, it is metabolized and the cells emit visible light. The IC detects this light, amplifies and filters this signal, and then reports this measurement. This measurement could be reported to the patient (*e.g.*, to a wristwatch receiver) or could be reported to an insulin pump in a closed-loop system that functions much like the pancreas.

FIG. 1 shows a perspective view of the present invention. Glucose **10** that is being detected enters the BBIC **11** through the semi-permeable membrane **12** that covers the bioreporter.

FIG 2 shows a side view of the present invention. The BBIC is enclosed in a biocompatible housing **20** with a semi-permeable membrane **21** covering the bioreporter held in a container **22**. The cells constituting the bioreporter may be in suspension or encapsulated in a polymer matrix. The bioreporter is separated from a photodetector **23** by a protective coating **24**. A single substance **25** contains the photodetector as well as additional circuits **26** that process and transmits the signal.

FIG. 3 shows a block diagram of one embodiment of the integrated circuit ("IC"). The photodetector is a photodiode **33** connected to a current to frequency converter **30**. The photodiode responds to light by sinking a current. The current is converted to a series of pulses that are accumulated in a digital counter **31**. The number of counts in the

counter in a fixed amount of time is directly proportional to the amount of light collected by the photodiode, which is directly proportional to the concentration of glucose. Digital processing circuitry in the digital counter would determine the appropriate next step for an insulin pump based on the measured glucose levels. The measured concentration or next instruction for the insulin pump could be reported *via* the wireless transmitter **32**. All these circuits (photodiode, signal processing, and wireless transmission) can be fabricated on one IC.

FIG. 4 shows the bioreporter being supplied with water and nutrients. A fluid and nutrient reservoir **141** is connected to a microfluidic pump **142** so that nutrient and fluid **144** may flow through the polymer matrix **143** enclosing the bioreporter. Each of these components can be constructed on a single substrate **140**.

FIG. 5A shows a high-quality photodetector made using a standard N-well CMOS process. The photodetector consists of two reverse biased diodes in parallel. The top diode is formed between the P+ active layer **45** and the N-well **46**, and the bottom diode is formed between the N-well **46** and the P-substrate **47**. The top diode has good short wavelength light sensitivity (400 – 550 nm), while the bottom diode provides good long wavelength sensitivity (500 – 1100 nm). Thus, the complete diode is sensitive over the range from 400 to 1100 nm. The luminescent compound under test **41** is separated from the photodetector by a layer **40** of Si_3N_4 and a layer **42** of SiO_2.

FIG. 10A, FIG. 10B, and **FIG. 10C** show schematic representations of an implantable biosensor containing two separate photodetectors with the bioreporters responding to either an increase or decrease in glucose concentrations.

FIG. 10B shows a side view of biosensor showing silastic covering.

FIG. 10C shows a schematic representaion of the utilization of a selectably permeable membrane to protect bioreporters from the immune response.

4.2 PHOTODETECTOR

The first element in the micro-luminometer signal processing chain is the photodetector. The key requirements of the photodetector are:

- Sensitivity to wavelength of light emitted by the bioluminescent or chemiluminescent compound under test;

- Low background signal (*i.e.* leakage current) due to parasitic reverse biased diodes;
- Appropriate coating to prevent the materials in the semiconductor devices from interfering with the bioluminescent or chemiluminescent process under study and to prevent the process under study from degrading the performance of the micro-luminometer; and,
- Compatibility with the fabrication process used to create the micro-luminometer circuitry.

Two photodetector configurations that satisfy these requirements are described below. It should be understood, however, that alternative methods of constructing such a photodetector can be used by one skilled in the art without departing from the spirit and scope of the invention as defined in the claims.

In the first embodiment, the photodetector is fabricated in a standard N-well CMOS process. Shown in **FIG. 5A**, this detector is formed by connecting the PN junction between the PMOS active region and the N-well in parallel with the PN junction between the N-well and the P-type substrate. The resulting detector is sensitive to light between approximately 400 nm and approximately 1100 nm, a range that encompasses the 450 to 600 nm emission range of most commonly used bioluminescent and chemiluminescent compounds or organisms. In order to meet the requirement that the device have a low background signal, the device is operated with a zero bias, setting the operating voltage of the diode equal to the substrate voltage. The photodiode coating may be formed with a deposited silicon nitride layer or other material compatible with semiconductor processing techniques.

In the second photodetector embodiment, the detector is fabricated in a silicon-on-insulator (SOI) CMOS process. The internal leakage current in an SOI process is two to three orders of magnitude lower than in standard CMOS due to the presence of a buried oxide insulating layer between the active layer and the substrate. Two photodetector structures are envisioned in the SOI process. The first structure, shown on the left of **FIG. 5B**, consists of a lateral PIN detector where the P-layer is formed by the P+ contact layer, the I (intrinsic) region is formed by the lightly doped active layer, and the N region is formed by the N+ contact layer of the SOI CMOS process. The spectral sensitivity of this

lateral detector is set by the thickness of the active layer. which may be tuned for specific bioluminescent and chemiluminescent compounds.

The second structure, shown on the right side of **FIG. 3B**, is similar to the first except that the junction is formed with a Schottky junction between a deposited cobalt silicide ($CoSi_2$) or other appropriate material layer and the lightly doped active layer.

The inventors contemplate that other photodetector configurations may be envisioned in silicon or other semiconductor processes meeting the criteria set forth above.

4.3 Low Noise Electronics

The low noise electronics are the second element in the micro-luminometer signal processing chain. The requirements for the low noise electronics are:

- Sensitivity to very low signal levels provided by the photodetector;
- Immunity to or compensation for electronic noise in the signal processing chain;
- Minimum sensitivity to variations in temperature;
- Minimum sensitivity to changes in power supply voltages (for battery powered applications);
- For some applications the electronics must have sufficient linearity and dynamic range to accurately record the detected signal level; and,
- In other applications the electronics must simply detect the presence of a signal even in the presence of electronic and environmental noise.

Three embodiments that satisfy these requirements are described below. It should be understood, however, that alternative methods of detecting small signals while satisfying these requirements may be used without departing from the spirit and scope of the invention as defined in the claims.

FIG. 6A schematically shows the first approach to the detection of very small signals. This device uses a P-diffusion/N-well photodiode, a structure compatible with standard CMOS IC processes, in the open circuit mode with a read-out amplifier (fabricated on the same IC with the photodiode). The luminescent signal generates electron-hole pairs in the P-diffusion and the N-well. The photo-generated electrons in the P-diffusion are injected into the N-well, while the photo-generated holes in the N-well

are injected into the P-diffusion. The N-well is tied to ground potential so that no charge builds up in this region. However, since the P-diffusion is only attached to the input impedance of a CMOS amplifier (which approaches infinity at low frequencies), a positive charge collects in this region. Thus, the voltage on the P-diffusion node begins to rise.

As the P-diffusion voltage begins to rise, the P-diffusion/N-well photodiode becomes forward biased, thereby producing a current in a direction opposite to the photo-generated current. The system reaches steady state when the voltage on the P-diffusion node creates a forward bias current exactly equal in magnitude (but opposite in polarity) to the photocurrent. If this PN junction has no deviations from the ideal diode equation, then the output voltage is given by the following equation:

$$V_{out} = V_t \ln(I_p / (A\, I_s) + 1), \qquad \text{(Eq. 1)}$$

where V_t is the thermal voltage (approximately 26 mV at room temperature), I_p is the photo-current, A is the cross-sectional area of this PN junction, and I_s is the reverse saturation current for a PN junction with unit cross-sectional area. The value of I_s depends greatly on the IC process and material parameters.

Two major error currents are present in PN junctions operating at low current density: recombination current and generation current. Except at very low temperatures, free carriers are randomly created in the PN junction space charge region. Since this region has a high field, these thermally excited carriers are immediately swept across the junction and form a current component (generation current) in the same direction as the photocurrent. Carriers crossing the space-charge region also have a finite chance of recombining. This creates another current component (recombination current) in the opposite direction of the photocurrent. Therefore, taking into account these error currents, Eq. 1 becomes:

$$V_{out} = V_t \ln((I_p + I_g - I_r) / (A\, I_s) + 1). \qquad \text{(Eq. 2)}$$

This output voltage is a function of parameters that are generally beyond the inventors' control. However, the inventors do have control over the junction area, A. Unfortunately, to make the inventors' output signal larger, the inventors want a small A, while the inventors want a large A for a high quantum efficiency (QE).

FIG. 6B shows a second microluminometer embodiment that satisfies both of these needs. This circuit uses a large area photodiode for efficient light collection, but uses a small-area diode in a feedback loop to supply the forward bias current that cancels out the photocurrent. Once again, the amplifier and feedback diodes are fabricated on the same IC as the photodiode. For this circuit:

$$V_{out} = 3\ V_t \ln((I_p + I_g - I_r) / (A_{fb} I_s) + 1), \qquad \text{(Eq. 3)}$$

where A_{fb} is the small cross-sectional area of the feedback diode. More than one diode is used in the feedback path to make the output signal large compared to the DC offset of any subsequent amplifier stages. This technique allows efficient collection of the light with a large-area photodiode, yet produces a large output voltage because of the small-area diodes in the feedback path.

The feedback circuit of **FIG. 6B** maintains the photodiode at zero bias. With no applied potential, the recombination and generation currents should cancel. Eq. 3 becomes:

$$V_{out} = 3\ V_t \ln((I_p / (A_{fb} I_s)) + 1) \qquad \text{(Eq. 4)}$$

if the smaller recombination and generation currents in the smaller feedback diodes are neglected.

The principal advantages of the second micro-luminometer embodiment shown in **FIG. 6B** include:

- The SNR is totally determined by the photodiode; noise from the small diode and amplifier are negligible;
- Diodes can be added in the feedback path until the signal level at the output of the amplifier is significant compared to offset voltages (and offset voltage drift) of subsequent stages;
- This method is completely compatible with standard CMOS processes with no additional masks, materials, or fabrication steps;
- This detection scheme can be fabricated on the same IC with analog and digital signal processing circuits and RF communication circuits; and,
- Measurement can be made without power applied to the circuit. Power must be applied before the measurement can be read, but the measurement can be obtained with no power.

A third microluminometer implementation shown in **FIG. 6C** uses correlated double sampling (CDS) to minimize the effects of low frequency (flicker) amplifier noise as well as time or temperature dependent variations in the amplifier offset voltage. As shown in **FIG. 6C**, a photodiode with capacitance C_d and noise power spectral density S_i is connected to an integrating preamplifier with feedback capacitance C_f and input noise power spectral density S_v through a set of switches that are controlled by the logical level of a flip-flop output. When the flip-flop output is low, the switches are positioned so that the photocurrent flows out of the preamplifier, causing the output voltage of the integrator to increase. When the low-pass filtered integrator output voltage exceeds a threshold, V_{HI}, the upper comparator "fires" setting the flip-flop and causing its output to go high. The detector switches change positions, causing current to flow into the integrating amplifier, which in turn causes the amplifier output voltage to decrease. When the integrator output goes below a second threshold, V_{LO}, the lower comparator "fires" resetting the flip-flop and causing the output to go low again. The process repeats itself as long as a photocurrent is present.

The average period of the output pulse, Δt, is given by the following equation:

$$\Delta t = \frac{2C_f(V_{HI} - V_{LO})}{I_P}, \qquad \text{(Eq. 5)}$$

where V_{HI} and V_{LO} are the threshold voltages of the comparators and I_p is the diode photocurrent. Two noise sources contribute to error in the measured value of Δt. S_i is the input noise current power spectral density associated primarily with the photodiode, and S_v is the input noise voltage power spectral density associated primarily with the preamplifier. The diode noise is given by the equation:

$$S_i = 2q(2I_s + I_p) \quad \left(\frac{A^2}{Hz}\right), \qquad \text{(Eq. 6)}$$

where I_s is the photodiode reverse saturation current and I_p is the photocurrent. As the photocurrent approaches zero, the noise power spectral density approaches a finite value of $4qI_s$ A^2/Hz. The noise voltage S_v of the preamplifier is determined by its design and has units of V^2/Hz.

The transfer function from the point where the diode noise is introduced to the output of the integrator is given approximately by the equation:

$$H_i(\omega) \approx \left(\frac{1}{sC_f}\right)\left(\frac{\omega_1}{s+\omega_1}\right), \qquad \text{(Eq. 7)}$$

where ω_1 is the corner frequency of the integrating amplifier and $s = j\omega$. Ignoring for the moment the effect of the switches, the transfer function from the point where the amplifier noise is introduced to the output of the integrator is given approximately by the equation:

$$H_v(\omega) \approx \left(\frac{C_f + C_d}{C_f}\right)\left(\frac{\omega_1}{s+\omega_1}\right). \qquad \text{(Eq. 8)}$$

The switches perform a correlated double sampling function that attenuates the noise that appears below the switching frequency of the output pulse string. The transfer function of a correlated double sampling circuit is approximated to first order by the equation:

$$H(\omega) \approx \left(\frac{s}{s+2/\Delta t}\right), \qquad \text{(Eq. 9)}$$

where Δt is the average period of the output pulse string. Thus, taking into account the switches, the transfer function from the point where the amplifier noise is introduced to the output of the integrator is approximately given by the equation:

$$H_v(\omega) \approx \left(\frac{C_f + C_d}{C_f}\right)\left(\frac{\omega_1}{s+\omega_1}\right)\left(\frac{s}{s+2/\Delta t}\right). \qquad \text{(Eq. 10)}$$

This is an important result because the effective zero introduced in the noise voltage transfer function reduces the effect of the flicker noise of the amplifier. This is particularly useful in CMOS implementations of the micro-luminometer where flicker noise can have a dominant effect.

The mean squared output noise at the output of the integrator is given by the equation:

$$v_n^2 = \int_{-\infty}^{\infty} S_v\left(H_v * H_v\right) + S_i\left(H_i * H_i\right) d\omega\,, \qquad \text{(Eq. 11)}$$

and the RMS noise voltage is then given by the equation:

$$\sigma_v = \sqrt{v_n^2}\,. \qquad \text{(Eq. 12)}$$

The RMS error in the measured period is determined by the slope of the integrated signal and the noise at the output of the integrator following the relationship:

$$\sigma_t = \frac{\sigma_v}{dv/dt} \qquad \text{(Eq. 13)}$$

or, approximately, by the equation:

$$\sigma_t \approx \frac{\sigma_v}{\frac{(V_{HI} - V_{LO})}{\Delta t}}. \qquad \text{(Eq. 14)}$$

The error in measuring Δt may be reduced by collecting many output pulses and obtaining an average period. The error in the measured average pulse period improves proportionately to the square root of the number of pulses collected, such that

$$\bar{\sigma}_t \approx \frac{\sigma_v}{\frac{(V_{HI} - V_{LO})}{\Delta t}} \frac{1}{\sqrt{N}} \qquad \text{(Eq. 15)}$$

or

$$\bar{\sigma}_t \approx \frac{\sigma_v}{\frac{(V_{HI} - V_{LO})}{\Delta t}} \sqrt{\frac{t_{meas}}{\Delta t}} \qquad \text{(Eq. 16)}$$

where t_{meas} is the total measurement time.

Thus, implementation of the micro-luminometer has the following advantages:

- The low frequency "flicker" noise of the amplifier is reduced by a correlated double sampling process; and,
- Ideally, the accuracy of the measured photocurrent may be improved without limit by acquiring data for increasing periods of time.

Of course, practical limitations imposed by the lifetime and stability of the signals produced by the luminescent compound under test will ultimately determine the resolution of this implementation.

4.4 READ-OUT ELECTRONICS

Several methods of communicating data from the BBIC to external receivers or *in vivo* drug delivery systems are envisaged. In a preferred embodiment the communication

method is an on-chip wireless communication system that reports the level of the photocurrent to computing circuitry contained within *in vivo* drug delivery system or an external receiver. In a closed-loop system, this computing circuitry would determine the amount of drug to be delivered by the *in vivo* drug delivery system. If an external receiver were used, the data from the BBIC along with the user inputs would be used to determine the amount of drug to be administered. The external receiver may include wireless transmission circuitry for communication with the *in vivo* drug delivery system or the drugs may be administered manually. Other methods of communicating BBIC data include;

- Generation of a DC voltage level proportional to the photocurrent with a hardwire connection to an *in vivo* drug delivery system;
- Generation of a DC current level proportional to the photocurrent with a hardwire connection to an *in vivo* drug delivery system;
- Generation of a logical pulse string whose rate is proportional to the photocurrent with a hardwire connection to an *in vivo* drug delivery system;
- On-chip implementation of an analog to digital converter that reports a numerical value proportional to the photocurrent with a hardwire connection to an *in vivo* drug delivery system;
- On-chip implementation of a serial or parallel communications port that reports a number proportional to the photocurrent with a hardwire connection to an *in vivo* drug delivery system;
- Generation of a logical flag when the photocurrent exceeds a predefined level with a hardwire connection to an *in vivo* drug delivery system; and,
- Generation of a radio-frequency signal or beacon when the photocurrent exceeds a predefined level.

Wireless communication *in vivo* may be limited by signal attenuation by body fluids, tissues, and health-related limits on RF signal levels. This may require the BBIC and *in vivo* drug delivery system to be closely spaced, which may not be the optimum configuration for all cases. In such cases, the BBIC could communicate to an external receiver located *ex vivo* but closer to the BBIC. This receiver could be connected

(hardwired or wirelessly) to a transmitter located *ex vivo* but closer to the *in vivo* drug delivery system.

Numerous algorithms are envisioned for controlling an *in vivo* drug delivery system with a BBIC. These include, but are not limited to

- a simple look-up table that administers a prescribed drug level that is determined only by a single BBIC data point;
- a simple look-up table that administers a prescribed drug level when a predetermined number of data points exceed a preset threshold;
- an algorithm that determines drug dosage by rate of increase or decrease of BBIC signal
- an algorithm that determines drug dosage by matching BBIC data points to data point patterns stored in memory
- learning algorithms that use BBIC data point history and user inputs to predict correct drug dosage to achieve desired results

Some of these algorithms may require two-way communication between the BBIC and *in vivo* drug delivery system. In this case, a receiver would be included on the BBIC.

4.5 Biocompatible Housing and Semi-Permeable Membrane

The BBIC is enclosed in a biocompatible housing with a semi-permeable membrane covering the bioreporter region. The preparation of biocompatible coverings for implants and prosthetic devices so as to minimize capsule formation and physiological rejection has been an area of extensive investigation. For example, U. S. Patent 5,370,684 and U. S. Patent 5,387,247 (each specifically incorporated herein by reference in its entirety), describe the application of a thin biocompatible carbon film to prosthetic devices. A biocompatible implant material comprising a three-dimensionally woven or knitted fabric of organic fibers is disclosed in U. S. Patent 5,711,960, specifically inlcuded nerein in its entirety. Other coverings for implants constructed to present a biocompatible surface to the body are described in U. S. Patent 5,653,755, U. S. Patent 5,779,734, and U. S. Patent 5,814,091 (each specifically incorporated herein by reference in its entirety). In addition, collagen coating and albumin coating have been shown to improve the biocomapatibilty of implants and prosthetic devices (Marios *et al.*, 1996; Ksander, 1988).

The present invention contemplates the use of any suitable biocompatible material to either coat or form the housing.

A semi-permeable membrane comprises that part of the BBIC housing that covers the bioreporter and entraps them on the integrated circuit. This membrane allows the selected substance, such as glucose, to pass to the bioreporter, yet prevents the passage of larger molecules. Membranes designed for use with glucose-oxidase based biosensors may also used in the preferred embodiments of the present invention. Membranes investigated and designed for use with glucose-oxidase based biosensors include, but are not limited to: polytetrafluoroethylene membranes (Vaidaya and Wilkins, 1993); perfluorinated ionomer membranes (Moussy *et al.*, 1994); charged and uncharged polycarbonate membranes (Vadiya and Wilkins 1994); and cellulose acetate membranes (Wang and Yuan, 1995; Sternberg *et al.*, 1988). In addition, other membranes have been developed for the use transplantation of islets or other cells bioengineered to produce insulin. The membranes must be permeable to glucose and other metabolites while exclude elements of the host immune system. Such membranes may be adapted for use with the present invention and include, but are not limited to: asymmetric poly(vinyl alcohol) membranes (Young *et al.*, 1996); poly(L-lysine) membranes (Tziampazis and Sambanis, 1995); ploy urethane (Zondervan *et al.*, 1992); nucleopore membranes (Ohgawara *et al.*, 1998) ; and agarose gel (Taniguchi *et al.*, 1997). Biocompatible semi-permeable membranes for encapsulation of cells to form an artificial organ are described in U. S. Patent 5,795,790 and U. S. Patent 5,620,883 (each specifically incorporated herein by reference in its entirety). A biocompatible semi-permeable segmented block polyurethane copolymer membrane and its use for permeating molecules of predetermined molecular weight range are disclosed in U.S. Patent No. 5,428,123, (specifcally incorporated herein by reference in its entirety). The present invention contemplates the use of any suitable semi-permeable membrane that allows the selected substance access to the bioreporter yet prevents the passage of larger molecules.

4.6 DRUG DELIVERY DEVICES

Numerous drug delivery devices, implantable and external, have been previously described which can be controlled by radio telemetry. For example, U. S. Patent 4,944,659 (specifically incorporated herein by reference in its entirety), describes an

implantable piezoelectric pump for drug delivery in ambulatory patients. U. S. Patent 5,474,552, specifically included herein in its entirety, describes an implantable pump for use in conjunction with a glucose sensor that can deliver multiple active agents, such as glucose, glucagon, or insulin as required. Separate pumps may be used for delivering each of the agents or a single pump that is switchable between them may be used. U. S. Patent 5,569,186, specifcally inlcluded herein in its entirety, describes a closed loop infusion pump system controlled by a glucose sensor. U. S. Patent 4,637,391, specifically included herein in its entirety, describes a remote controlled implantable micropump for delivery of pharmaceutical agents. The use of external drug delivery systems is contemplated in other embodiments of the present invention. For example, U. S. Patent 5,800,420, specifically inlcuded herein in its entierty, discloses a pump position topically against the skin surface that delivers a liquid drug, such as insulin, *via* a hollow delivery needle extending into the dermis. In other embodiments of the present invention, the drug delivery system may be interfaced with the biosensor device and controlled directly, as opposed to remote telemetry control, from the BBIC.

The pump delivery systems described above are examples to facilitate the use of the present invention. Drug delivery devices other than pump systems are also contemplated by the present invention. For example, U. S. Patent 5,421,816, specifcially included herein in its entirety, describes an ultrasonic transdermal drug delivery system. Ultrasonic energy is used to release a stored drug and forcibly move the drug through the skin of an organism into the blood stream. Thus the invention contemplates the use of any suitable drug delivery system that can be controlled by the BBIC glucose monitor. The factors dictating the choice of such a drug delivery system and its use with the BBIC glucose monitor use will be known to those of skill in the art in light of the present disclosure.

4.7 BIOLUMINESCENT BIOREPORTERS

In a preferred embodiment of the invention, the bioreporter for glucose monitoring will be a mammalian bioluminescent reporter cell line that has been genetically engineered to express luminescence in response to glucose concentrations on a continuos basis. An implantable bioluminescent sensor requires a bioluminescent reporter that can function without the exogenous addition of substrate for the luciferase reaction. Current

eukaryotic luciferase systems used in molecular biology require the addition of exogenous substrate because of the complex nature for the production of eukaryotic luciferins. Cells must be either permeabilized or lysed and then treated with an assay solution containing luciferin. Thus current eukaryotic luciferases systems are not preferred candidates for on-line monitoring.

The requirement for the addition of exogenous substrate can be obviated by the use of bacterial *lux* genes. In a preferred embodiment of the present invention the *lux* genes of *X. luminescens*, *luxAB* and *luxCDE*, are used as the bioluminescent reporter system. The *X. luminescens luxAB* gene encodes the α- and β-subunits of a luciferase enzyme that exhibits greatest thermostability at 37°C, while other bacterial luciferases lose significant activity above 30°C. The *luxCDE* genes are required to eliminate the need for the addition of exogenous substrate. The aldehyde substrate of the luciferase encoded by the *luxAB* genes is generated by a fatty acid reductase complex coded for by the *luxCDE* genes. The preferred fatty acid for this reaction is myristic acid, which is present in eukaryotic organisms (Rudnick *et al.*, 1993), and thus eukaryotic cells are suitable host cells for this reporter. The enzyme complex reduces the fatty acid to the corresponding aldehyde. The luciferase then oxidizes the aldehyde to back to the fatty acid.

Other bioluminescence nucleic-acid segments may include the *lux* genes of *Vibrio fischerii*, *luxCDABE*, or luciferases from other organisms capable of bioluminescence that can be adapted so not as to require the addition of exogenous substrate. In other embodiments of the invention, nucleic acid segment encodes green fluorescent protein of *Aqueorea victoria* or *Renilla reniformis*.

4.8 Recombinant Vectors Expressing Bioluminescence Genes

One important embodiment of the invention is a recombinant vector that comprises one or more nucleic-acid segments encoding one or more bioluminescence polypeptides. Such a vector may be transferred to and replicated in a eukaryotic or prokaryotic host.

It is contemplated that the coding DNA segment will be under the control of a recombinant, or heterologous promoter. As used herein, a recombinant or heterologous promoter is intended to refer to a promoter that is not normally associated with a DNA segment encoding a crystal protein or peptide in its natural environment. Naturally, it will

be important to employ a promoter that effectively directs the expression of the DNA segment in the cell type, organism, or even animal, chosen for expression. The use of promoter and cell type combinations for protein expression is generally known to those of skill in the art of molecular biology (*see e.g.*, Sambrook *et al.*, 1989). In a preferred embodiments of this. such promoters are directed by *cis*-acting glucose response elements. In one preferred embodiment, the glucose response element is the L4 box which directs the L-pyruvate kinase ("L-PK") promoter in liver and islet β-cells. The L4 box consists of a tandem repeat of non-canonical E-boxes (Kennedy *et al.*. 1997). Glucose enhances the hepatic and pancreatic β-cell by modifying the transactivating capacity of upstream stimulatory factors ("USF") bound to the L4 box (Kennedy *et al.*, 1997; Doiron *et al.*, 1996).

The exact mechanism by which glucose controls the transactivational capacity of USF proteins is unclear. One possibility is the reversible phosphorylation of USF proteins. Glucose may alter the phosphorylation status through the pentose phosphate shunt *via* xyulose 5-phosphate (Dorion *et al.*, 1996). An alternative mechanism is *via* the intracellular concentration of glucose 6-phosphate (Foufelle *et al.*, 1992). Other glucose metabolites may also be implicated. Phosphorylated glucose metabolites include, but are not limited to, fructose 6-phosphate, 6-phosphogluconic acid, 6-phosphoglucono-δ-lactone, ribulose 5-phosphate, ribose 5-phosphate, erythrose 4-phosphate, sedoheptulose 7-phosphate, glyceraldehyde 3-phosphate and dihdyroxyacetone phosphate. Non-phosphorylated glucose metabolites include, but are not limited to, citric acid, *cis*-aconitic acid, *threo*-isocitric acid, succinic acid, fumaric acid, malic acid, oxaloacetic acid, pyruvic acid and lactic acid.

Another glucose response element, similar in arrangement to the L-PK gene L4 box, is the regulatory sequence involved in the transcriptional induction of the rat S14 gene (Shih *et al.*, 1995). Other glucose response elements that have been described include, but are not limited to, the hepatic 6-phosphofructo-2-kinase gene (Dupriez and Rousseau, 1997), the β-islets insulin gene (German and Wang, 1994), the mesangial transforming growth factor-beta gene (Hoffman *et al.*, 1998), and the gene for acetyl-coenzyme-A carboxylase (Girard *et al.*, 1997). The present invention contemplates the use any glucose response element that can effectively direct a promoter or otherwise control the expression of the reporter protein in response to glucose.

In a preferred embodiment, the recombinant vector comprises a nucleic-acid segment encoding one or more bioluminescence polypeptides. Highly preferred nucleic-acid segments are the *lux* genes of *X. luminescens luxAB* and *luxCDE*. Bacterial luciferases may have to be modified to optimize expression in eukaryotic cells. Almashanu *et al.* (1990) fused the *luxAB* genes from *V. harveyi* by removal of the TAA stop codon from *luxA*, the intervening region between the two genes, and the initial methionine from *luxB* without disrupting the reading frame. The fusion was successfully expressed in *Saccharomyces cerevisiae* and *Drosophila melanogaster*. The same strategy was used with *luxAB* from *X. luminescens*. The resultant construct has been sequenced to verify the genetic changes to generate the fusion and they were confirmed. The sequence of the fusion region is as follows:

5'-tacctagggagaaagagaatg-3' (SEQ ID NO:7)

(end of *luxA* underlined) (start of *luxB* underlined)

The fusion successfully expresses fused protein in *E.coli* and has been successfully cloned into the mammalian vector as described in Section 5.1.2.

In a further embodiment, the inventors contemplate a recombinant vector comprising a nucleic-acid segment encoding one or more enzymes that are capable of producing a reaction that yields a luminescent product or a product that can be directly converted to a luminescent signal. For example, substrates of the commonly used β-galactosidase and alkaline phosphates enzymes are commercially available that are luminescent (chemiluminescence) when converted by the respective enzyme.

In another important embodiment, the biosensor comprises at least a forst transformed host cell that expresses one or more of recombinant expression vectors. The host cell may be either prokaryotic or eukaryotic. In a preferred embodiment, the host cell is a mammalian cell. Host cells may include stem cells, β-islets cells or hepatocyte cells. In a preferred embodiment the host cells are homologous cells, *i.e.* cells taken from the patient that are cultured, genetically engineered and then incorporated in the BBIC. Particularly preferred host cells are those which express the nucleic-acid segment or segments comprising the recombinant vector which encode the *lux* genes of *X. luminescens, luxAB* and *luxCDE*. These sequences are particularly preferred because the transcribed proteins of the *X. luminescens lux* system have the ability to function at 37°C (ambient human body temperature).

A wide variety of ways are available for introducing a nucleic-acid segment expressing a polypeptide able to provide bioluminescence or chemiluminescence into the microorganism host under conditions that allow for stable maintenance and expression of the gene. One can provide for DNA constructs which include the transcriptional and translational regulatory signals for expression of the nucleic-acid segment, the nucleic-acid segment under their regulatory control and a DNA sequence homologous with a sequence in the host organism, whereby integration will occur or a replication system which is functional in the host, whereby integration or stable maintenance will occur or both.

The transcriptional initiation signals will include a promoter and a transcriptional initiation start site. In preferred instances, it may be desirable to provide for regulative expression of the nucleic-acid segment able to provide bioluminescence or chemiluminescence, where expression of the nucleic-acid segment will only occur after release into the proper environment. This can be achieved with operators or a region binding to an activator or enhancers, which are capable of induction upon a change in the physical or chemical environment of the microorganisms. For translational initiation, a ribosomal binding site and an initiation codon will be present.

Various manipulations may be employed for enhancing the expression of the messenger RNA, particularly by using an active promoter, as well as by employing sequences, which enhance the stability of the messenger RNA. The transcriptional and translational termination region will involve stop codon or codons, a terminator region, and optionally, a polyadenylation signal (when used in an Eukaryotic system).

In the direction of transcription, namely in the 5′ to 3′ direction of the coding or sense sequence, the construct will involve the transcriptional regulatory region, if any, and the promoter, where the regulatory region may be either 5′ or 3′ of the promoter, the ribosomal binding site, the initiation codon, the structural gene having an open reading frame in phase with the initiation codon, the stop codon or codons, the polyadenylation signal sequence, if any, and the terminator region. This sequence as a double strand may be used by itself for transformation of a microorganism host, but will usually be included with a DNA sequence involving a marker, where the second DNA sequence may be joined to the expression construct during introduction of the DNA into the host.

By "marker" the inventors refer to a structural gene that provides for selection of those hosts that have been modified or transformed. The marker will normally provide for selective advantage, for example, providing for biocide resistance (*e.g.*, resistance to antibiotics or heavy metals); complementation, so as to provide prototrophy to an auxotrophic host and the like. One or more markers may be employed in the development of the constructs, as well as for modifying the host.

Where no functional replication system is present, the construct will also include a sequence of at least 50 basepairs (bp), preferably at least about 100 bp, more preferably at least about 1000 bp, and usually not more than about 2000 bp of a sequence homologous with a sequence in the host. In this way, the probability of legitimate recombination is enhanced, so that the gene will be integrated into the host and stably maintained by the host. Desirably, the nucleic-acid segment able to provide bioluminescence or chemiluminescence will be in close proximity to the gene providing for complementation as well as the gene providing for the competitive advantage. Therefore, in the event that the nucleic-acid segment able to provide bioluminescence or chemiluminescence is lost, the resulting organism will be likely to also have lost the complementing gene, and the gene providing for the competitive advantage, or both.

A large number of transcriptional regulatory regions are available from a wide variety of microorganism hosts, such as bacteria, bacteriophage, cyanobacteria, algae, fungi, and the like. Various transcriptional regulatory regions include the regions associated with the *trp* gene, *lac* gene, *gal* gene, the λ_L and λ_R promoters, the *tac* promoter. See for example, U. S. Patent 4,332,898; U. S. Patent 4,342,832; and U. S. Patent 4,356,270 (each specifically incorporated herein by reference in its entirety). The termination region may be the termination region normally associated with the transcriptional initiation region or a different transcriptional initiation region, so long as the two regions are compatible and functional in the host. In a preferred embodiment of the present invention, a fragment of the L-pyruvate kinase gene is used that contains the L-PK promoter and the L4 box glucose responsive elements as described by Kennedy *et al.* (1997). In a highly preferred embodiment, the p.LPK.Luc_{FF} plasmid is used (Kennedy *et al.*, 1997), with the exception that the *luc* gene coding for the firefly luciferase is removed and replaced with the fused *X. luminescens luxAB* genes.

Where stable episomal maintenance or integration is desired, a plasmid will be employed which has a replication system that is functional in the host. The replication system may be derived from the chromosome, an episomal element normally present in the host or a different host, or a replication system from a virus that is stable in the host. A large number of plasmids are available, such as pBR322, pACYC184, RSF1010, pR01614, and the like. See for example, Olsen *et al.*, 1982; Bagdasarian *et al.*, 1981, and U. S. Patent 4,356,270, U. S. Patent 4,362,817, U. S. Patent 4,371,625, and U. S. Patent 5,441,884, each of which is incorporated specifically herein by reference.

The desired gene can be introduced between the transcriptional and translational initiation region and the transcriptional and translational termination region, so as to be under the regulatory control of the initiation region. This construct will be included in a plasmid, which will include at least one replication system, but may include more than one, where one replication system is employed for cloning during the development of the plasmid and the second replication system is necessary for functioning in the ultimate host. In addition, one or more markers may be present, which have been described previously. Where integration is desired, the plasmid will desirably include a sequence homologous with the host genome.

The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired organism as against unmodified organisms or transferring organisms, when present. The transformants then can be tested for bioluminescence or chemiluminescence activity. If desired, unwanted or ancillary DNA sequences may be selectively removed from the recombinant bacterium by employing site-specific recombination systems, such as those described in U. S. Patent 5,441,884, specifically incorporated herein by reference in its entirety.

4.9 ASSEMBLY AND STORAGE OF THE *IN VIVO* BIOSENSOR

When the biosensor consists of bioengineered cells entrapped in suspension behind a semi-permeable membrane, as opposed to encapsulated in a matrix, the cells may be added to the BBIC any time from immediately to several h before implantation of the biosensor. The biosensor may alternatively consist of cells encapsulated in a polymeric matrix. Matrices will include materials previously shown to be successful in the encapsulation of living cells, including polyvinyl alcohol, sol-gel, and alginate (Cassidy *et*

al., 1996). Prior to encapsulation, prokaryotic cell lines may be lyophilized in a freeze dry system (*e.g.*, Savant) following the manufacturer's protocol. Lyophilization allows cells to undergo periods of long-term storage (several years) with a simple rehydration protocol being required for cell resuscitation prior to BBIC use (Malik *et al.*, 1993). *S. cerevisiae* eukaryotic cells may be similarly lyophilized. Eukaryotic cell lines, preferably consisting of islet β-cells, stem cells, or hepatic cells, may be encapsulated on the IC within polyvinyl alcohol mesh-reinforced or microporous filter supported hydrogels, which have previously been successfully implemented in these types of cell encapsulations (Baker *et al.*, 1997; Burczak *et al.*, 1996; Gu *et al.*, 1994; Inoue *et al.*, 1991).

In the case of the mammalian cell lines, lyophilization, however, is not an alternative. In such cases, mammalian cells may be encapsulated in a sol gel or another immobilization matrix as previously described and attached to the BBIC. The completed BBIC in its enclosure would then be stored in serum or another appropriate maintenance medium and maintained until use. The advantage of using an immortal stem cell line is apparent for both long-term use and storage. Implantation may be performed according to the specific application. In the case of glucose detection, an area where interstitial fluid is accessible would be most appropriate. However an implantable device with the specific application of detecting hormones or other blood borne molecules would have to be accessible to the bloodstream. A synthetic vein or catheter system may need to be employed to allow continuous monitoring of the blood levels of the target molecule. A specific example other than glucose would be the use of the *in vivo* biosensor device to detect molecules associated with colon cancer. In this case the biosensor would be implanted in the colon.

Integrated circuits may be individually packaged in sterile, static-proof bags. Prokaryotic-based and yeast eukaryotic biosensors consisting of lyophilized cells may be individually stored in sterile, static-proof, vacuum sealed bags for time periods approaching several years. Cells typically undergo rehydration in a minimal nutrient medium prior to use. Mammalian cell systems will remain frozen for long-term storage (up to 7 years at -150°C) or refrigerated for short-term storage (several days), either separately or, if entrapped, frozen or refrigerated *in situ* on the BBIC. In all cases, cell viability may be checked be exposing the BBIC to a known concentration of the analyte of interest, thus producing a quantitative bioluminescent signal of known magnitude. One

or more control vials of analyte(s) or reference "standards" may be included as part of a diagnostic kit, or may be supplied for proper calibration of the implantable device.

4.10 Implantation and Use of the Biosensor Devices

In a preferred embodiment of the present invention, the BBIC analyte biosensor is implanted such that it is contact with the interstitial fluid of the animal. For example, in the case of glucose biosensors, it has been shown that glucose kinetics in interstitial fluid can be predicted by compartmental modeling (Gastaldelli *et al.*, 1997). In particular the subcutaneous placement of glucose sensors has been demonstrated (Schmidt *et al.*, 1993; Poitout *et al.*, 1993; Ward *et al.*, 1994; Stenberg *et al.*, 1995; Bantle and Thomas, 1997). Other potential analyte biosensor tissue implant sites include the peritoneum, pleura and pericardium (Wolfson *et al.*, 1982). In fact, the inventors contemplate that depending upon the particular analyte or metabolite that is being detected, the implantable biosensor may be placed in any convenient location throughout the body using conventional surgical and implant methodologies. For example, the device may be implanted in such as way as to be in contact with interstitial fluid, lymph fluid, blood, serum, synovial or cerebrospinal fluid depending upon the particular analyte to be detected.

In certain embodiments the implantable device msy be placed in contact with particular tissues, organs, or particular organ systems. Likewise, it may be desirable to implant the biosensor such that it contacts particular intracellular fluids, intercellular fluids, or any other body fluid in which the target substance can be monitored.

The present invention also contemplates the use of multiple biosensors for the detection of a plurality of different analytes. For example, in the case of glucose monitoring, one or more devices may be used to monitor various glucose or glucose metabolites, glucagons, insulin, and the like. Likewise, one or more biosensor devices may be employed in controlled drug delivery systems. As such, the device may be operably connected to a drug delivery pump or device that is capable of being controlled by the biosensor and that is able to introduce into the body of the animal an amount of a particular drug, hormone, protein, peptide, or other pharmaceutical composition determined by the concentration of one or more analytes detected by the BBIC device. Thus, controlled drug delivery systems are contemplated by the inventors to be particularly desirable in providing long-term administration of drugs to an animal such as

in the case of chronic or life-long medical conditions or where symptoms persist for a long period of time. The long term controlled delivery of drugs such as pain medications, heart or other cardiac regulators, diuretics, or homones or peptides such as insulin, or metabolites such as glucagon or glucose can be facilitated by such biosensor/pump systems. In cases where it is necessary to deliver more than one drug or metabolite to the animal, multiple drug delivery systems or a single switchable drug delivery system is contemplated to be particularly useful.

Host-rejection effects can be minimized through immunoisolation techniques. Previous studies have shown that living non-host cells enclosed in hydrogel membranes are protected from immune rejection after transplantation (Baker *et al.*, 1997; Burczak *et al.*, 1996; Inoue *et al.*, 1991). The hydrogels block access by the humoral and cellular components of the host's immune system but will remain permeable to the target substance glucose. A mesh-reinforced polyvinyl alcohol hydrogel bag developed by Gu *et al.* (1994) may be used to fully encapsulate the BBIC, allowing for transplantation void of immunosuppressive responses.

Host rejection of the implanted biosensor is not an issue if cells from the host are used for the biosensor construction. However if other cell lines are used it may be necessary to provide a barrier between the cells and the appropriate body fluid that permits passage of the signature molecules or analytes but not bioreporter cells or body cells (white blood cells, *etc.*). Immunosuppressed patients are not affected, as the implant does not contain any kind of pathogenic agent that would affect the patient. In all cases, the surgical methods involved in implantation of the disclosed BBIC devices are well known to one of skill in the surgical arts.

In an illustrative embodiment, the BBIC glucose sensor may be used for monitoring glucose in diabetic patients. However, such a sensor can also be used in other conditions where glucose concentrations are of concern, such as in endurance athletes or other condition involving either hypo- or hyperglycemia. Such measurements may be the end point for investigative or diagnostic purposes or the sensors may be linked *via* telemetry or directly to a drug delivery system.

The use of implantable BBICs for substances other than glucose can be used in a range of therapeutic situations. With the incorporation of an appropriate *cis*-activating response element, BBICs could monitor a number of substances and could find use in

chronic pain treatment, cancer therapy, chronic immunosuppression, hormonal therapy, cholesterol management, and lactate thresholds in heart attack patients. For example, Section 5.7 describes the use of the BBICs in the detection and diagnosis of cancer.

Individual biosensors can be calibrated to check for viability of the cells as well as performance. The calibration is performed by exposing the sensor to solutions containing varying concentrations of the analyte(s) of interest. The bioreporter may be calibrated by a series of standard analyte concentrations for the specific application after its initial construction. The overall on-line performance can be monitored using microfluidics with a reservoir of the analyte, which would systematically provide a known concentration to the cells this would allow both calibration and test for viability.

The luminescence response is then correlated to concentration and the parameters set. Viability can also be continuously monitored by bioengineered cells in which the reporter exhibits continuous luminescence. Loss of viability results in decreased luminescence. This technique has been used to detect the viability of prokaryotic cells. Thus the BBIC would contain two bioreporters, the bioreporter detecting the selected substance and the second bioreporter exhibiting a luminescence proportional to cell viability. Measurement of the ratio of the signals from the two bioreporters would give a detection method that would automatically correct for any loss in viability.

Once prepared the bioreporters can be stored in the appropriate maintenance medium (*e.g.*, standard tissue culture media, sera, or other suitable growth or nutrient formulations), and then calibrated prior to implantation. The viability of the devices may be checked by bioluminescence using microfluidics, or by the quantiation of known standards or other reference solutions to ensure viability and integrity of the system prior to, or after implantation..

In certain embodiments of the invention, the monolithic biosensor devices may be used external to the body of the monitored individual. In some clinical settings the monitor may be used to monitor glucose in body fluids in an extracorporeal fashion. The device may even be used in the pathological or forensic arts to detect the quantity of particular analytes in body tissues or fluids and the like. Likewise, the present invention also contemplates use of the biosensor devices in the veterinary arts. Implantation of such devices in animals for the monitoring of hormone levels in the blood (*i.e.* for optimizing milk production), monitoring the onset of estrous (heat) in numerous animals to maximize

artificial insemination efficiency, and monitoring hormone levels in the milk produced on-line (in the udder) *etc.* is contemplated to provide particular benefits to commercial farming operations, livestock industries and for use by artisans skilled in veterinary medicine.

4.11 DIAGNOSTICS KITS COMPRISING *IN VIVO* BIOSENSORS

While the individual components of the invention described herein may be obtained and assembled individually, the inventors contemplate that, for convenience, the components of the biosensor may be packaged in kit form. Kits may comprise, in suitable container means, one or more bioreporters and an integrated circuit including a phototransducer. The kit will also preferably contain instructions for the use of the biosensor apparatus, and may further, optionally comprise a drug delivery device or a second biosensor apparatus. The kit may comprise a single container means that contains one or more bioreporters and the integrated circuit including a phototransducer and drug delivery device. Alternatively, the kits of the invention may comprise distinct container means for each component. In such cases, one container would contain one or more bioreporters, either in an appropriate medium or pre-encapsulated in a polymer matrix, another container would include the integrated circuit, and another conatiner would include the drug delivery device. When the bioreporter is pre-encapsulated, the kit may contain one or more encapsulation media. The use of distinct container means for each component would allow for the modulation of various components of the kits. For example, several bioreporters may be available to choose from, depending on the substance one wishes to detect. By replacing the bioreporter, one may be able to utilize the remaining components of the kit for an entirely different purpose, thus allowing reuse of components.

The container means may be a container such as a vial, test tube, packet, sleeve, shrink-wrap, or other container means, into which the components of the kit may be placed. The bioreporter or any reagents may also be partitioned into smaller containers or delivery vehicles, should this be desired.

The kits of the present invention also may include a means for containing the individual containers in close confinement for commercial sale, such as, *e.g.,* injection or blow-molded plastic containers into which the desired components of the kit are retained.

Irrespective of the number of containers, the kits of the invention also may comprise, or be packaged with, an instrument for assisting with the placement of the bioreporter upon the integrated circuit. Such an instrument may be a syringe, pipette, forceps, or any other similar surgical or implantation device. The kit may also comprise one or more stents, catheters, or other surgical instrument to facilitate implantation within the body of the target animal. Such kits may also comprise devices for remote telemetry or devices for data storage or long term recordation of the data obtained from the monitoring device. Likewise, in the case of controlled drug delivery systems, the kits may comprise one or more drug delivery pumps as described above, and may also comprise one or more pharmaceutical agents themselves for administration. As an example, in the case of a glucose monitoring system, the system would typically comprise a glucose-sensitive BBIC device, a drug delivery pump, instructions for the implantation and/or use of the system, and optionally, reference standards or pharmaceutical formulations of insulin, glucagon or other pharmaceutical composition. The system may also optionally comprise growth and/or storage medium to support the nutritive needs of the bioreporter cells comprised within the BBIC device.

5.0 EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

5.1 EXAMPLE 1 -- CONSTRUCTION OF A BIOLUMINESCENCE REPORTER FOR MAMMALIAN CELL LINES

To facilitate the construction of an implantable bioluminescent glucose sensor it will be necessary to create a bioluminescent reporter system that can function without the exogenous addition of substrate for the luciferase reaction. This exogenous addition is

due to the complex nature of the production of luciferins for the various eukaryotic luciferases. Cells must be either permeablized or lysed and then treated with an assay solution containing luciferin. Therefore, the present state of bioluminescence reporters used in eukaryotic molecular biology makes them unsuitable for "on-line" monitoring. The firefly luciferase has been used in examining the regulation of L-pyruvate kinase promoter activity in single living rat islet β-cells (Kennedy *et al.*, 1997). However, these cells had to be perfused with Beetle luciferin in order to generate a luminescence response.

To alleviate this limitation, a preferred bioluminescent reporter system for the present invention is one that does not require the addition of exogenous substrate. In the case of bacterial luciferase-based detection systems, this may be accomplished using the bioluminescent genes from *X. luminescens.* In this organism, *luxA* and *luxB* genes (or a single fused *luxAluxB* gene encode the α- and β-subunits, respectively, of the luciferase enzyme (Meighen *et al.*, 1991). This luciferase exhibits greatest thermostability at 37°C while other bacterial luciferases lose significant activity above 30°C. Therefore, these bacterial luciferases can be expressed in eukaryotic cells with slight modification. Almashanu *et al.* (1990) fused the *luxAB* genes from *V. harveyi* by removal of the TAA stop codon from *luxA*, the intervening region between the two genes, and the initial methionine from *luxB* without disrupting the reading frame. The fusion was successfully expressed in *S. cerevisiae* and *D. melanogaster.* Using the same strategy a fused *luxAB* gene sequence was developed using the genes from *X. luminescens.*

To eliminate the need for the addition of exogenous substrate, cells must themselves supply the appropriate substrate for the luciferase. In the bacterial system the substrate is generated by a fatty acid reductase complex encoded by the *luxCDE* genes. This enzyme complex reduces short chain fatty acids to the corresponding aldehyde. The luciferase then oxidizes the aldehyde to the corresponding fatty acid. The preferred fatty acid for this reaction is myristic acid, which is present in eukaryotic organisms (Rudnick *et al.*, 1993). Myristic acid is usually involved in the myristoylation of the amino terminus that is associated with membrane attachment (Borgese *et al.*, 1996, Brand *et al.*, 1996). Thus, to obviate the need for an exogenous supply of the luciferase substrate, the biosensor also preferably comprises a nucleic acid sequence that encodes the three *luxC*, *luxD*, and *luxE*-encoded subunits. As in the case of the luxAluxB gene fusion, the *luxC*,

luxD, and *luxE* genes have been fused to produce a single *luxCDE* gene fusion that encodes the three subunits of the enzyme complex. The methods of preparing such gene fusions are described below:

5.1.1 FUSION OF THE *LUXAB* AND *LUXCDE* GENES

The *luxAB* genes may be fused using conventional molecular biology techniques. For example, the polymerase chain reaction may be routinely employed for this purpose. By synthesizing a 5'-primer whose sequence begins with ATG for the start codon for the *luxA* gene juxtaposed by a 3'-primer ending with the codon immediately preceding the ATT stop codon. These primers may then be used in amplification reactions and the product gel purified. The *luxB* gene may also be amplified as above using primers that eliminate the ATG initial methionine codon but preserve the reading frame. The PCR™ reactions employ a thermostable polymerase such as the Pfu™ polymerase of Stratagene (La Jolla, CA), which does not have terminal deoxytransferase activity and therefore generates a blunt end. The resultant PCR™ products are blunt-end ligated, and the ligation is then subjected to PCR™ using the 5'-primer from *luxA* and the 3'-primer from *luxB* using Taq polymerase to facilitate TA cloning (Invitrogen, San Diego, CA). Only ligations with the correct orientation of fragments are amplified. The *luxAB* amplicon is then gel purified and TA cloned into a suitable vector (such as the PCRII™ vector) and transformed into *E. coli* using standard manufacturer's protocols.

Transformants are screened for light production by the addition of *n*-decanal which, when oxidized by the luciferase, generates bioluminescence. Only colonies emitting light are selected since they are in the proper orientation for further genetic manipulation. The *luxCDE* fusion is generated using the same strategy as above except transformants are screened by minipreps followed by restriction digestion analysis to determine orientation. Plasmids are amplified in *E. coli*, recovered and purified twice on CsCl gradients.

5.1.2 EXPRESSION OF *LUXAB* AND *LUXCDE* IN HELA CELLS

To determine the relative activity of the fused bacterial luciferase components, cloned fragments containing *luxAB* are cloned into a suitable mammalian expression vector (such as pcDNA 3.1 and *luxCDE*-containing fragments are cloned into a suitable

mammalian expression vector (such as pcDNA/Zeo 3.1) (Invitrogen, Faraday, CA). Both vectors constitutively express inserted genes. HeLa cells are then transfected with *luxAB* or both *luxAB* and *luxCDE* and selected using appropriate antibiotics following the manufacturer's protocol (Promega, Madison, WI). Cells receiving the *luxAB* fusion are exposed to *n*-decanal and checked for bioluminescence. These cells cotransfected with *luxCDE* are then examined for bioluminescence to ascertain the relative expression of the *luxCDE* fusion. This permits the comparison of bioluminescence *via* the addition of exogenous aldehyde versus aldehyde that is produced endogenously.

An alternate strategy to enhance bioluminescent expression involves engineering a vector that would contain three copies of the eukaryotic expression machinery contained in pcDNA3.1 (Stratagene, La Jolla, CA). This allows for the expression of the individual components of *luxCDE* since it has already been shown that the fused luciferase is expressed in eukaryotic cell lines (Almashanu *et al.*, 1990).

5.2 Example 2 – Construction of a Glucose Bioluminescent Biosensor

The firefly luciferase has been used in examining the regulation of L-pyruvate kinase promoter activity in single living islet β-cells (Kennedy *et al.*, 1997). A glucose response element designated the L4 box has been determined to be in the proximal promoter. A 200-bp fragment containing this region was cloned in front of the firefly luciferase (*luc*) in plasmid pGL3Basic resulting in a glucose reporter plasmid designated p.LPK.Luc_{FF}. Results resulted in the detection of single cells that were exposed to 16 mM glucose but not 3 mM glucose. However, these cells had to be perfused with Beetle luciferin making it unacceptable for an on-line biosensor. Therefore, a bioluminescent sensor for glucose was constructed by replacing the firefly luciferase in p.LPK.Luc_{FF} with the fused *luxAB* gene as described below.

5.3 Example 3 – Bioluminescent Reporter Construction and Transfection of Rat Islet β-Cells

The bioluminescent reporter plasmid was constructed by removing the *luc* gene coding for the firefly luciferase from p.LPK.Luc_{FF} and replacing it with the fused *luxAB* gene. This was accomplished by cleaving the *luc* gene from p.LPK.Luc_{FF} and cloning in

the *luxAB* gene. The resultant plasmid was amplified in *E. coli* and the plasmid DNA extracted and double purified on CsCl gradients.

Islet cells were prepared as previously described (German *et al.*, 1990) and transfected by electroporation with the bioluminescent reporter construct and the plasmid containing the constitutively expressed *luxCDE* construct. This configuration causes the cells to maintain a pool of the aldehyde substrate that is available to the reporter genes *(luxAB)*. Cells were screened for light production in a range of glucose concentrations from 3 mM to 30 mM. Transfected cells were washed, concentrated, and placed in a microwell in a light-tight cell that is then affixed to the integrated circuit. Different concentrations of glucose and assay media (Kennedy *et al.*, 1997) were added to the cells to examine sensitivity and response time of the glucose BBIC.

5.4 EXAMPLE 4 – PREPARATION OF BIOLUMINESCENT REPORTER CONSTRUCTS

The use of reporter gene technology is widespread in studying gene regulation in both eukaryotic and prokaryotic systems. Various genes are used depending on the cell lines being investigated. However with the BBIC technology the use of reporter genes that result in the emission of light is required. Therefore, reporter genes coding for bioluminescence are utilized. All previously developed reporters utilizing other reporter genes for example the gene coding for β-galactosidase (*lacZ*) may be converted to the bioluminescent version using standard molecular techniques and the reporter genes utilized in this specific application (modified *lux* system). Therefore, any currently existing reporter cell line for testing gene expression in mammalian cell lines may be adapted for use as a bioreporter when converted to the *lux* reporter. The implantable system simply contains the appropriate reporter cell line. Table 1 shows a list of examples of eukaryotic reporter cell lines that may be exploited in an implantable biosensor.

TABLE 1

Reporter Gene Fusion	Application	Reference
ADH4-*LUC*	Monitors expression of alcohol dehydrogenase to increasing concentrations of alcohol	Edenberg *et al.*, 1999
TH-*lacZ*	Shows increased gene expression in mice subjected to chronic cocaine or morphine exposure	Boundy *et al.*, 1998
Estrogen regulated-*LUC*	Detects estrogens and xenoestrogens by there effect on the estrogen response element	Balaguer *et al.*, 1999
IGFBP-5-*LUC*	Detects the presence of progesterone by the upregulation of the reporter construct	Boonyaratanakornkit *et al.*, 1999
CYP1A-*lacZ*	Detects compounds that cause an upregulation of cytochrome P450 (potential carcinogens)	Campbell *et al.*, 1996

5.5 EXAMPLE 5 -- CONSTRUCTION AND IMPLANTATION OF A GLUCOSE BIOSENSOR AND INSULIN DELIVERY PUMP

In one embodiment, a pair of bioluminescent reporters may be utilized that are in tandem and that specifically respond to deviations in glucose concentrations. One bioreporter utilizes the *luxAB* and *luxCDE* genes from *X. luminescens* incorporated into a plasmid-based system designated p.LPK.Luc$_{FF}$, which contains a eukaryotic *luc* gene able to respond to glucose concentrations (increasing bioluminescence corresponds to increasing glucose concentrations). The second bioreporter utilizes a plasmid construct containing the promoter for the phosphoenolpyruvate carboxylase gene (PEPCK) that also responds to glucose concentrations, except increased bioluminescence corresponds to decreased levels of glucose. The incorporation of the *luxAB* and *luxCDE* genes into each construct allow for bioluminescence measurements to occur in real-time with deviations in glucose concentrations, negating the requirement for cell destruction and substrate addition.

In this embodiment, the integrated circuit comprises separate photodetector units for each bioreporter (FIG. 9A, FIG. 9B, and FIG. 9C). Bioluminescent responses from each construct can be independently monitored, allowing for the signal processing circuitry to differentiate between one bioreporter's response to increased glucose concentrations and the second bioreporter's response to decreased glucose concentrations.

The signal processing circuitry processes the signals from the photodetectors, converts it to a digital format and relays the information to the implanted insulin pump (FIG. 10). The tandem set of bioreporters allows a more accurate signal as well as redundancy in the detector. Due to the often-fatal outcome of hypoglycemia, this tandem system also allows for more careful monitoring and warning of the onset of hypoglycemia.

The cells used in the tandem bioreporter system may be affixed to each of the photodetectors either directly by attachment or encapsulated in hydrogel (Prevost *et al.*, 1997). It may be necessary to isolate the bioreporters using a semi-permeable membrane to allow the transport of small molecules such as glucose and insulin across the membrane and prohibit the influx of immune effector cells and antibodies (Monaco *et al.*, 1993, Suzuki *et al.*, 1998). However small molecules such as cytokines can still enter the selective membranes and interfere with the bioluminescent reporter cell lines. This approach has been used extensively by those of skill in the art.

When applicable, bioluminescent reporter cell lines may be constructed from cells taken directly from the patient to receive the implant. This approach is particularly desirable in cases of long-term implants such as implantable insulin delivery. Cells may be obtained from the patient, genetically engineered for the appropriate monitoring function, grown in cell culture, evaluated and then preserved for long-term storage. The use of cell lines developed from the patient's own cells, is particularly desirable as it reduces the chance of host rejection and creation of an immune response to the implanted device. Preferably, stem cells (immortal stem cells, if attainable) are used when appropriate, and may be maintained and nourished in suitable culture medium. Such pluripotent, totipotent, or otherwise immortal cell lines provide particular advantage in the creation of suitable long-term implantable devices.

Before implantation the biosensor may be calibrated injecting the chamber containing the cells with various concentrations of glucose delivered from an auxiliary pump and reservoir on the insulin delivery pump (FIG. 10). This permits determination of the appropriate parameters to allow the proper dosage of insulin to be delivered. Once the parameters are set, the pump may be evaluated for insulin delivery. Systematically the glucose biosensor is recalibrated in the patient utilizing the glucose standard contained in the delivery pump.

In the case of drug delivery systems, the glucose biosensor may be operably connected to the delivery pump *via* a hardwire or wireless connection. The biochip provides digital data that may be input directly to the signal processing circuitry of the pump to proportionally dispense the insulin. Alternatively, the digital data may be converted into analog data and used to control the pump. When a wireless capability is added to the bioreporter device, remote monitoring of the sensor is possible. For example, in this configuration, the patient may place a radio transmitter/receiver outside the body near the implanted device to communicate the data from the implanted device toa remote station. In some applications, the radio transmitter/receiver may be linked to a computer programmed to forward the data to a remote station over a network such as a local area network, a wide area netword, or even the Internet. Such wireless applications allow remote monitoring and maintenance of the patients. There are several pumps currently on the market, which are candidates for interfacing with the biosensor. In one embodiment, the Medtronic Synchronized infusion system may be used as it has extensively used in drug delivery and utilizes a portable computer to allow programming of the pump from outside the body (www.asri.edu/neuro/brochure/pain6.htm). The pump can also be refilled through the skin *via* a self-sealing septum. The pump is one inch thick and three inches in diameter and weighs approximately six ounces. The biosensor can be integrated into the preexisting electronic circuitry to take advantage of the out-of-body programming by a portable computer. The chip can be powered utilizing the battery that powers the delivery pump.

The biosensor/insulin pump apparatus may be surgically implanted using local anesthesia in the abdominal cavity. Both the sensor and the pump may be implanted in the peritoneal space of the abdomen both for simplicity and to avoid the complications of direct catheter placement in the blood stream. Glucose concentrations are monitored and the insulin delivered peritoneally as required by the patient (FIG. 10).

5.6 EXAMPLE 6 -- BIOLUMINESCENT REPORTER CONSTRUCTION AND TRANSFECTION OF RAT ISLET β-CELLS AND H4IIE HEPATOMA CELLS

The regulation of the PEPCK gene will be exploited in the construction of the bioluminescent reporter for detecting decreased glucose concentrations. This system is highly regulated as the phosphoenolpyruvate carboxylase is the rate-limiting enzyme in

gluconeogenesis. PEPCK gene expression is increased in the presence of glucocorticoids and cAMP and decreased in the presence of insulin (Sasaki *et al.*, 1984; Short *et al.*, 1986). In both rat liver and H4IIE hepatoma cells the insulin effect is dominant and the glucocorticoids and cAMP is additive. The promoter region of the PEPCK will be cloned in front of the fused *luxAB*. The resultant construct will then produce increased bioluminescence in the presence of low glucose concentrations.

The bioluminescent reporter plasmid for detecting increased glucose concentration may be constructed by removing the *luc* gene coding for the firefly luciferase from p.LPK.Luc_{FF} and replacing it with the fused *luxAB*. This is accomplished by cleaving the *luc* gene from p.LPK.Luc_{FF} and cloning in the *luxAB* gene. The bioluminescent reporter plasmid for the detection of low glucose concentrations is constructed by replacing the chloramphenicol transferase (CAT) gene in the previously constructed PEPCK promoter CAT fusion (Petersen *et al.*, 1988; Quinn *et al.*, 1988) with the *luxAB* gene. The resultant plasmid is amplified in *E. coli* and the plasmid DNA extracted and double purified on CsCl gradients.

Islet and hepatoma cells may be prepared as previously described (German *et al.*, 1990; Petersen *et al.*, 1988) and co-transfected with the bioluminescent reporter construct and the plasmid containing the constitutively expressed *luxCDE* gene constructed in objective one. This configuration causes the cells to maintain a pool of aldehyde substrate that will be available to the reporter genes *(luxAB)*. Cells are screened for light production in a range of glucose concentrations from 3 mM to 30 mM. Transfected cells are washed, concentrated, and placed in a microwell in a light-tight chamber that is then affixed to the integrated circuit. Different concentrations of glucose and assay media (Kennedy *et al.*, 1997) are added to the cells to examine sensitivity and response time of the glucose BBIC. After initial characterization. the bioluminescent glucose reporters may also be tested in a flow cell. Cells are placed in an encapsulation medium on the integrated circuit and media containing different concentrations of glucose (3-to-30 mM) is then perfused across the cells to examine dynamic responses.

5.7 EXAMPLE 7 – BBICS IN THE DIAGNOSIS AND DETECTION OF CANCER

Colon cancer is the second leading cause of cancer death after lung cancer in the United States, and the incidence increases with age in that 97% of colon cancer occurs in

persons greater than 40 (Coppola and Karl, 1998). Although most cases of colon cancer are sporadic, in 15% of the patients there is a strong familial history of similar tumors in first-degree relative relatives (Coppola and Karl, 1998). These familial cancers such as hereditary nonpolyposis colon cancer (HNPCC) and familial adenomatous polyposis (FAP) result from autosomal dominant inheritable genetic mutations in putative tumor suppressor genes, and a spectrum of lesions occurs from hyperplasia-dysplasia-adenoma-carcinoma (Coppola and Karl, 1998). Because much of the early molecular lesions are known about inherited colonic cancer, they represent a useful model for development of a novel biosensor strategy for early clinical detection. Biosensors are hybrid devices combining a biological component with a computerized measuring transducer.

This example describes the adaptation of the implantable biosensor device to permit early detection of cancers, and to permit means for monitoring remission and recurrence of cancer. Because the miniaturized biosensors of the present invention are small enough to be implantable, and can be combined with a reporter system engineered to produce light without the need for cellular lysis or additional substrate, a powerful tool for early diagnosis of colon cancer in the form of an implantable device is now possible for the first time.

As described above for glucose and other metabolite biosensors, the inducible reporter system utilized is based on the *luxAB* and *luxCDE* genes from *X. luminescens* placed in a eukaryotic reporter cell so that expression of certain genes or their products can be detected by expression of bioluminescence by the BBICdevice. The eukaryotic reporter cell is treated with mitomycin C so it is unable to divide, but is still able to respond metabolically and produce a quantitative bioluminescent signal.

Colon cancer is the second leading cause of cancer death in the United Sates, with at least 50% of the population developing a colorectal tumor by the age of 70 (Kinzler and Vogelstein, 1996). Although most cases of colorectal cancer are sporadic, 15% are the result of heritable cancer syndromes, familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) (Kinzler and Vogelstein, 1996). Familial adenomatous polyposis is a syndrome characterized by the development of hundreds to thousands of adenomas or polyps in the colon and rectum, only a small number of which develop into invasive cancer (Kinzler and Vogelstein, 1996). Loss of function of both alleles of the *adenomatous polyposis coli (APC)* tumor suppressor gene

predisposes persons to develop malignant cancer (Coppola and Marks, 1998). In addition, most sporadic colon cancers are also found to contain mutations in the *APC* gene (Kinzler and Vogelstein, 1996). In hereditary nonpolyposis colorectal cancer, there is marked microsatellite instability secondary to mutations in DNA mismatch repair genes such as *hMSH2* and *hMSH1*; single, high grade tumors develop at a young age and are usually confined to the right colon (Coppola and Karl, 1996; Smyrk, 1994). Whereas cells with mutations in *APC* are generally aneuploid from loss of whole sections of chromosomes, cells with mutations in *hMSH2* or *hMSH1* are euploid (Lengauer *et al.*, 1998).

The molecular events leading to the development of colonic neoplasia are fairly well understood (Kinzler and Vogelstein, 1996). Persons with complete loss of APC develop lesions in the colon called dysplastic aberrant crypt foci that progress to early adenomas (Kinzler and Vogelstein, 1996). Other mutations begin to accumulate, such as those in K-Ras or p53, and the tumors progress to late adenomas, carcinomas, and metastatic carcinomas (Kinzler and Vogelstein, 1996). A similar progression is seen in HNPCC as well. Because the sequence of genetic events is fairly well understood for both these types of cancer, they represent excellent models for development of sensitive and specific diagnostic tests that can be used to detect one or more altered cells *in vitro*.

The *APC* gene encodes a cytoplasmic protein that localizes to the ends of microtubules at focal adhesion complexes (Kinzler and Vogelstein, 1996). As cells migrate up through the crypts, expression of APC increases until the terminally differentiated and located colonic epithelial cells undergo apoptosis (Kinzler and Vogelstein, 1996). Cadherins are transmembrane proteins that are localized to focal adhesion plaques in most epithelial cells (Aplin *et al.*, 1998). The carboxy terminus of each cadherin interacts with cytoplasmic structural proteins known as catenins (Aplin *et al.*, 1998). There are three types of catenins: β-catenin binds to the cytoplasmic domain of cadherin; -catenin binds to β-catenin and the actin cytoskeleton via -actinin; -catenin functions in place of β-catenin in some cell types (Aplin *et al.*, 1998). β-Catenin also is part of a signal transduction pathway involving the secreted glycoprotein Wnt and glycogen synthase kinase 3 (GSK3) (Aplin *et al.*, 1998). APC interacts with several components of the Wnt-β-catenin-GSK3 pathway, including β- and γ-catenins, GSK3, and tubulin (Aplin *et al.*, 1998). Most of the mutations in colorectal cancer are in the carboxy terminal region of APC so that it can no longer bind β-catenin (Aplin *et al.*, 1998). In

fact, -catenin lies downstream of APC and is critical for its function as a tumor suppressor gene (Aplin *et al.*, 1998). When the Wnt pathway is inactivated, GSK3 phosphorylates the N-terminus of β-catenin, targeting it for degradation by the ubiquitin pathway (Munemitsu *et al.*, 1996). When β-catenin accumulates, it activates gene transcription via the transcription factor Lef-1/TCF (Morin *et al.*, 1997). APC works in concert with GSK3 to inhibit -catenin-mediated transcriptional activity (Kinzler and Vogelstein, 1996).

In hereditary nonpolyposis colon cancer, microsatellite instability is the result of mutations in one or more DNA-mismatch repair genes (Jiricny 1998; Nicholaides *et al.*, 1994). At least 90% of HNPCC tumors have microsatellite instability (Karran 1996; Smyrk 1994). One potential marker for microsatellite instability in colorectal tumors is inactivation of the type II receptor for TGF-β (Markowitz *et al.*, 1995). Loss of function of βRII is associated with loss of growth regulation and tumor progression in colorectal adenomas in HNPCC (Wang *et al.*, 1995). Other signaling components of the TGF-β pathway that are involved in colorectal tumorigenesis include mutations in Smad 3 and Smad 4, both of which result in the development of colorectal adenocarcinomas in mice (Zhu *et al.*, 1998; Takaku *et al.*, 1998). Loss of function of βRII is a useful marker for early lesions in HNPCC (Markowitz *et al.*, 1995).

Because mutations in *APC* are the most common mutations in colorectal cancer, a reporter construct for T cell transcription factor (Tcf) was devised to screen multiple colon cancer cell lines for activation of transcriptional activity. Mutations in either *APC* or β-catenin result in activation of Tcf-responsive transcription through the accumulation of unphosphorylated cytoplasmic β-catenin (Morin *et al.*, 1997) and detecting activation of a reporter construct is useful as a marker for mutations in either of these genes. The vector pDISPLAY (Invitrogen) permits expression of the promoter for Tcf on the surface of the bioreporter cell; this construct consists of a tandem set of Tcf promoters: one upstream of the genes for *luxAB*, the other upstream of the *luxCDE*. *In* the presence of excess β-catenin the promoter constructs will stimulate activity of the reporter and bioluminescence will result.

Once the HepG2 and HeLa cells have been transfected with pcDNA3 encoding the *luxAB* genes. the cells are attached to the biosensor chip. It is necessary to insure that these cells are incapable of dividing, so after transfection and selection, the cells are irradiated with 6,000 rads γ-radiation from a ^{60}Co source (UT College of Veterinary

Medicine). In some embodiment it may be necessary to attach the cells to the biochip prior to irradiation so that efficient attachment can occur. An alternative is to treat the cells with mitomycin C to prevent further mitosis. Biochips may be coated with Matrigel, a basement membrane material that promotes attachment of epithelial cells. An alternative approach suspends the cells in Matrigel and allows it to form a gel on the surface of the biochip. The cells are then immobilized in the basement membrane material and are not subject to dislodgement by friction. Optionally, the surface of the chip may be altered by adding a net charge (*e.g.*, poly-L-lysine), coating the surface with surgical tissue glue, or by adding some other surface modification that allows the biopolymers to adhere tightly to the surface. Because mutations in *APC* are the most common mutations in colorectal cancer, a reporter construct for T cell transcription factor (Tcf) may be devised to screen multiple colon cancer cell lines for activation of transcriptional activity.

The present invention also provides a biosensor that may be used for endoscopic screening of the colonic mucosa to detect the presence of mutated cells prior to the onset of gross morphological alterations. It may be necessary to attempt detection of more than one abnormality at a time for the degree of sensitivity needed to detect small foci of malignant transformation. For example, many colonic tumors, especially those with mutations in *APC*, overexpress cyclooxygenase-2 (COX-2) and secrete large amounts of prostaglandins (Kutchera *et al.*, 1996; Sheng *et al.*, 1997; Coffey *et al.*, 1997; Kinzler and Vogelstein, 1996). Cyclooxygenase-2 is an early response gene that not constitutively expressed, but is turned on in colonic epithelial cells by growth factors and tumor promoters (Kutchera *et al.*, 1996; Sheng *et al.*, 1997; Coffey *et al.*, 1997). It may be possible to bioengineer reporter cells to bioluminesce in the presence of increased levels of prostaglandins in the intestinal lumen. Prostaglandins freely pass the cell membrane and would be able to enter the cytoplasm of the reporter cell to activate a reporter construct. Engineering a reporter cell to detect increased levels of prostaglandins through the use of the cyclooxygenase-2 promoter fused to the *luxAB* genes could also be of benefit in early detection of colon cancer. Because the levels of prostaglandins may be elevated in inflammation as well as neoplasia, this approach lacks appropriate specificity for diagnosing cancer. It would, however, be useful in determining which patients would benefit from treatment with specific cyclooxygenase inhibitors.

6.0 REFERENCES

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All of the compositions, methods, devices, apparatus and systems disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the methods, devices, apparatus and systems of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods, devices, apparatus and systems and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims. Accordingly, the exclusive rights sought to be patented are as described in the claims below.

CLAIMS:

1. An implantable monolithic bioelectronic device for detecting an analyte within the body of an animal, said device comprising:

(a) a bioreporter atop a substrate on an integrated circuit, said bioreporter being capable of metabolizing said analyte and emitting light consequent to such metabolism when in contact with said analyte; and,

(b) a sensor closely positioned to said integrated circuit that generates an electrical signal in response to receiving said emitted light,

wherein said device is contained within a biocompatible container that is implanted within the body of said animal.

2. The implantable monolithic bioelectronic device of claim 1, wherein said biocompatible container comprises a polymeric matrix.

3. The implantable monolithic bioelectronic device of claim 2, wherein said polymeric matrix comprises polyvinyl alcohol, poly-L-lysine, or alginate.

4. The implantable monolithic bioelectronic device of claim 2, wherein said polymeric matrix further comprises a microporous, mesh-reinforced or filter-supported hydrogel.

5. The implantable monolithic bioelectronic device of claim 1, wherein said integrated circuit comprises a phototransducer.

6. The implantable monolithic bioelectronic device of claim 5, further comprising a transparent, biocompatible, bioresistant separator operably positioned between the phototransducer and the bioreporter.

7. The implantable monolithic bioelectronic device of claim 1, wherein said bioreporter comprises a plurality of eukaryotic or prokaryotic cells that produce a bioluminescent reporter polypeptide in response to the presence of said analyte.

8. The implantable monolithic bioelectronic device of claim 7, wherein said plurality of prokaryotic cells comprise bacteria.

9. The implantable monolithic bioelectronic device of claim 7, wherein said plurality of eukaryotic cells comprise mammalian cells.

10. The implantable monolithic bioelectronic device of claim 9, wherein said plurality of eukaryotic cells comprise islet β-cells, immortal stem cells, or hepatic cells.

11. The implantable monolithic bioelectronic device of claim 10, wherein said plurality of eukaryotic cells comprise recombinant human immortal stem cells.

12. The implantable monolithic bioelectronic device of claim 7, wherein said plurality of cells comprise a nucleic acid segment that encodes a luciferase polypeptide or a green fluorescent protein that is produced by said cells in response to the presence of said analyte.

13. The implantable monolithic bioelectronic device of claim 12, wherein said nucleic acid segment encodes an *Aqueorea victoria* or a *Renilla reniformis* green fluorescent protein.

14. The implantable monolithic bioelectronic device of claim 12, wherein said nucleic acid segment encodes a humanized green fluorescent protein.

15. The implantable monolithic bioelectronic device of claim 12, wherein said nucleic acid segment encodes a bacterial Lux polypeptide.

16. The implantable monolithic bioelectronic device of claim 15, wherein said nucleic acid segment encodes a bacterial LuxA, LuxB, LuxC, LuxD, or LuxE polypeptide, or a LuxAB, or LuxCDE fused polypeptide.

17. The implantable monolithic bioelectronic device of claim 16, wherein said nucleic acid segment encodes a *Vibrio fischerii* or a *Xenorhabdus luminescens* LuxA, LuxB, LuxC, LuxD, or LuxE polypeptide, or a LuxAB, or LuxCDE fused polypeptide.

18. The implantable monolithic bioelectronic device of claim 17, wherein said nucleic encodes a *Xenorhabdus luminescens* LuxA, LuxB, LuxC, LuxD, or LuxE polypeptide, or a LuxAB, or LuxCDE fused polypeptide.

19. The implantable monolithic bioelectronic device of claim 18, wherein said polypeptide is encoded by a sequence comprising at least 25 contiguous nucleotides from SEQ ID NO:1.

20. The implantable monolithic bioelectronic device of claim 19, wherein said polypeptide is encoded by a sequence comprising at least 30 contiguous nucleotides from SEQ ID NO:1.

21. The implantable monolithic bioelectronic device of claim 20, wherein said polypeptide is encoded by a sequence comprising at least 35 contiguous nucleotides from SEQ ID NO:1.

22. The implantable monolithic bioelectronic device of claim 16, wherein the expression of said nucleic acid segment is regulated by a nucleic acid sequence comprising a *cis*-acting element that is responsive to the presence of said analyte.

23. The implantable monolithic bioelectronic device of claim 22, wherein said *cis*-acting response element is a nucleotide sequence selected from the group consisting of an S14 gene sequence, a hepatic L-pyruvate kinase gene sequence, a hepatic 6-phosphofructo-2-kinase gene sequence, a β-islets insulin gene sequence, a mesangial transforming growth factor-β gene sequence, and an acetyl-coenzyme-A carboxylase gene sequence.

24. The implantable monolithic bioelectronic device of claim 23, wherein said *cis*-acting response element comprises a contiguous nucleotide sequence from a β-islets insulin gene sequence or a hepatic L-pyruvate kinase gene sequence.

25. The implantable monolithic bioelectronic device of claim 12, wherein expression of said nucleic acid sequence is regulated by a promoter sequence derived from an L-pyruvate kinase-encoding gene.

26. The implantable monolithic bioelectronic device of claim 1, wherein said analyte is glucose, glucagon or insulin.

27. The implantable monolithic bioelectronic device of claim 7, further comprising a source of nutrients capable of sustaining said cells.

28. The implantable monolithic bioelectronic device of claim 1, further comprising a wireless transmitter.

29. The implantable monolithic bioelectronic device of claim 1, further comprising an antenna.

30. The implantable monolithic bioelectronic device of claim 1, further comprising an implantable drug delivery pump capable of being controlled by said device, and capable of delivering said drug to the body of said animal.

31. The implantable monolithic bioelectronic device of claim 1, wherein said biocompatible container further comprises a membrane that is permeable to said analyte but not to said bioreporter.

32. The implantable monolithic bioelectronic device of claim 1, wherein said bioreporter expresses said light-emitting polypeptide following the metabolism of said analyte by said bioreporter.

33. The implantable monolithic bioelectronic device of claim 2, wherein said biocompatible container comprises silicon nitride or silicon oxide.

34. The implantable monolithic bioelectronic device of claim 1, wherein said integrated circuit is a complementary metal oxide semiconductor (CMOS) integrated circuit.

35. The implantable monolithic bioelectronic device of claim 5, wherein said phototransducer comprises a photodiode.

36. The implantable monolithic bioelectronic device of claim 1, wherein said integrated circuit further comprises a photodiode and a current to frequency converter.

37. The implantable monolithic bioelectronic device of claim 1, wherein said integrated circuit further comprises a current to frequency converter and a digital counter.

38. The implantable monolithic bioelectronic device of claim 1, further comprising a transmitter.

39. The implantable monolithic bioelectronic device of claim 38, wherein said transmitter is capable of transmitting digital data.

40. An implantable controlled drug delivery system, comprising the device of claim 1, and an implantable drug delivery pump capable of being operably controlled by said device.

41. A method of providing a controlled supply of a drug to a patient in need thereof, comprising implanting within the body of said patient the controlled drug delivery system of claim 40.

42. A method of determining the amount of a drug required by a patient in need thereof, comprising implanting within the body of said patient the device of claim 1, and determining the amount of drug required by said patient based upon the output from said device.

43. A kit for the detection of an analyte comprising the device of claim 1 and instructions for using said device.

44. The kit of claim 43, further comprising a standardized reference solution.

45. A method of regulating the blood glucose level of an animal in need thereof, comprising monitoring the level of glucose in the bloodstream or inerstitial fluid of said patient using the device of claim 1 or the kit of claim 43, and administering

to said patient an effective amount of an insulin composition sufficient to regulate said blood glucose level.

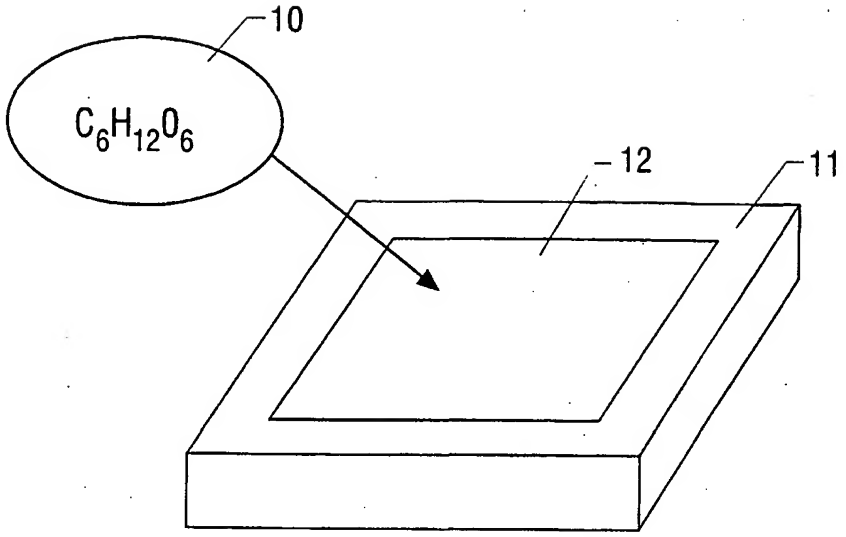


FIG. 1

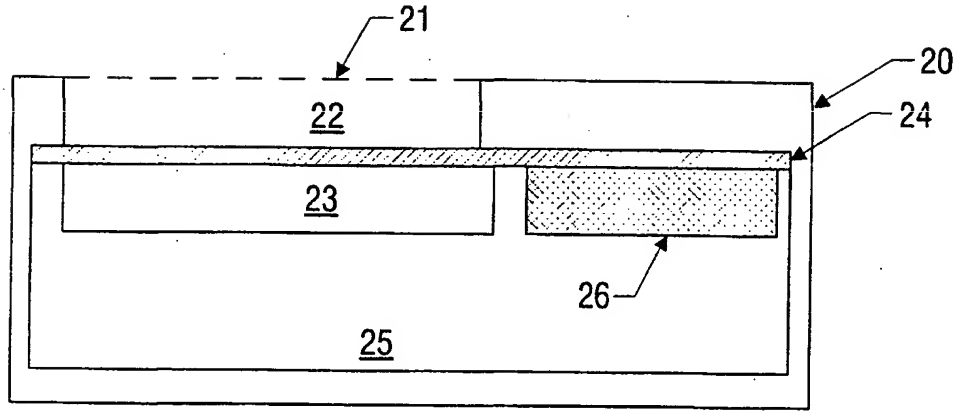
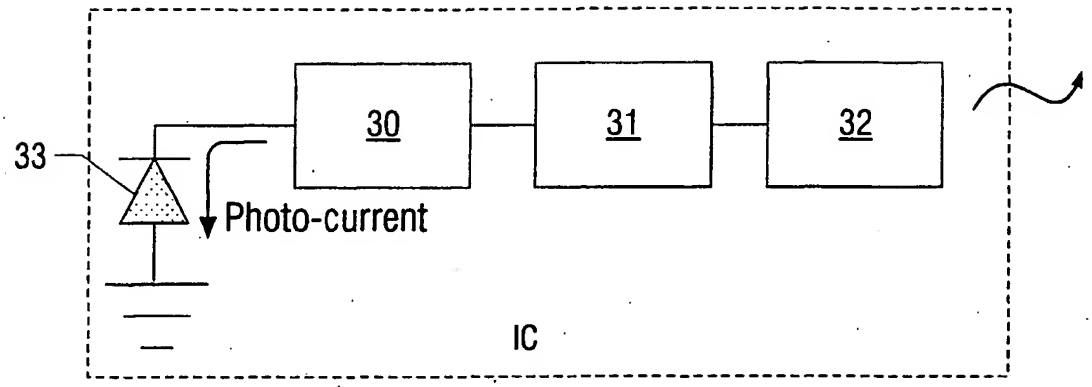


FIG. 2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IC

FIG. 3

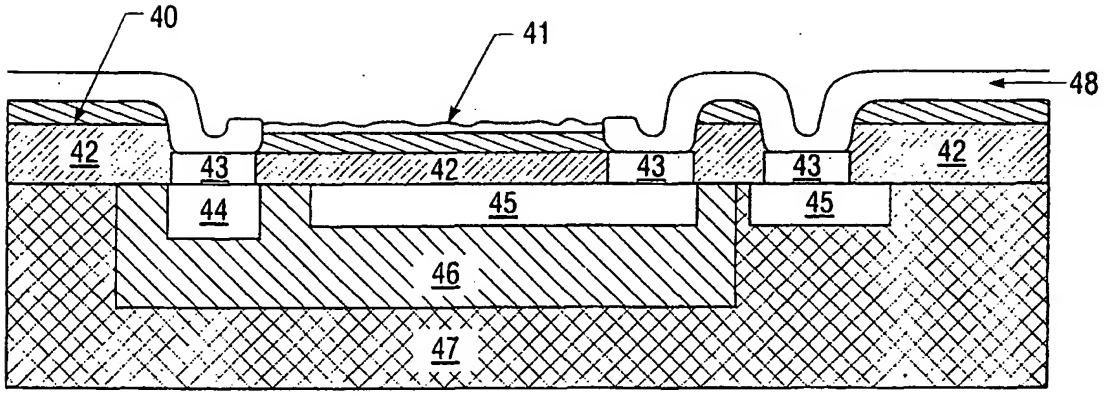
40
41
48
42
43
42
43
43
42
44
45
45
46
47

FIG. 4A

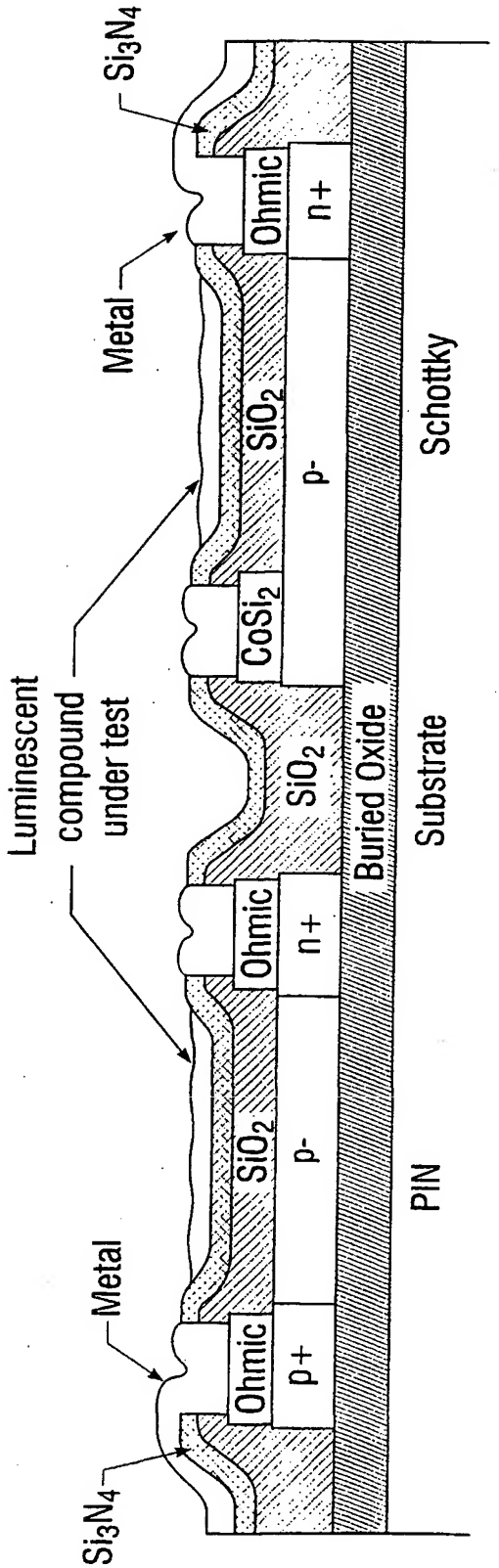
Si_3N_4
Metal
Ohmic
n+
SiO_2
p-
$CoSi_2$
Schottky
Luminescent compound under test
SiO_2
Buried Oxide
Substrate
Ohmic
n+
SiO_2
p-
PIN
Metal
Ohmic
p+
Si_3N_4

FIG. 4B

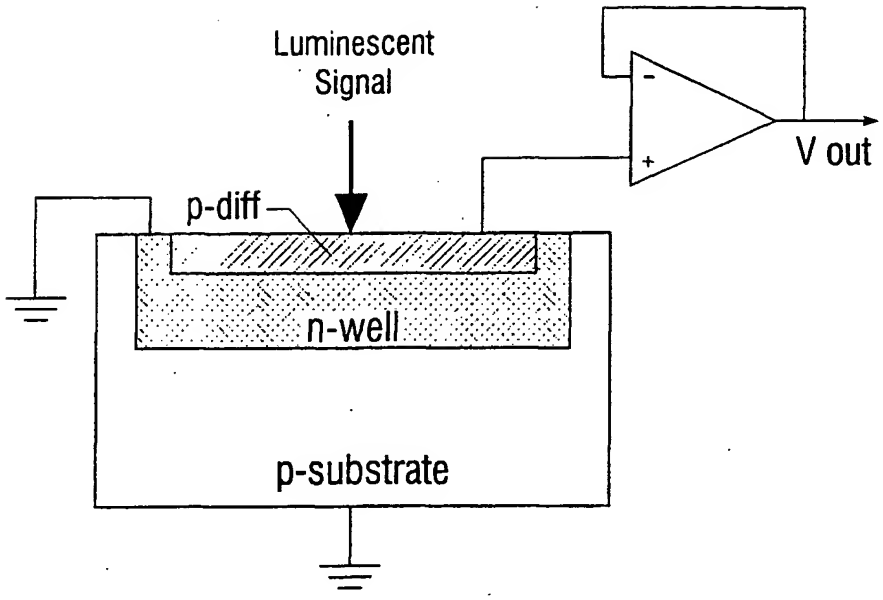
Luminescent
Signal
V out
p-diff
n-well
p-substrate

FIG. 5A

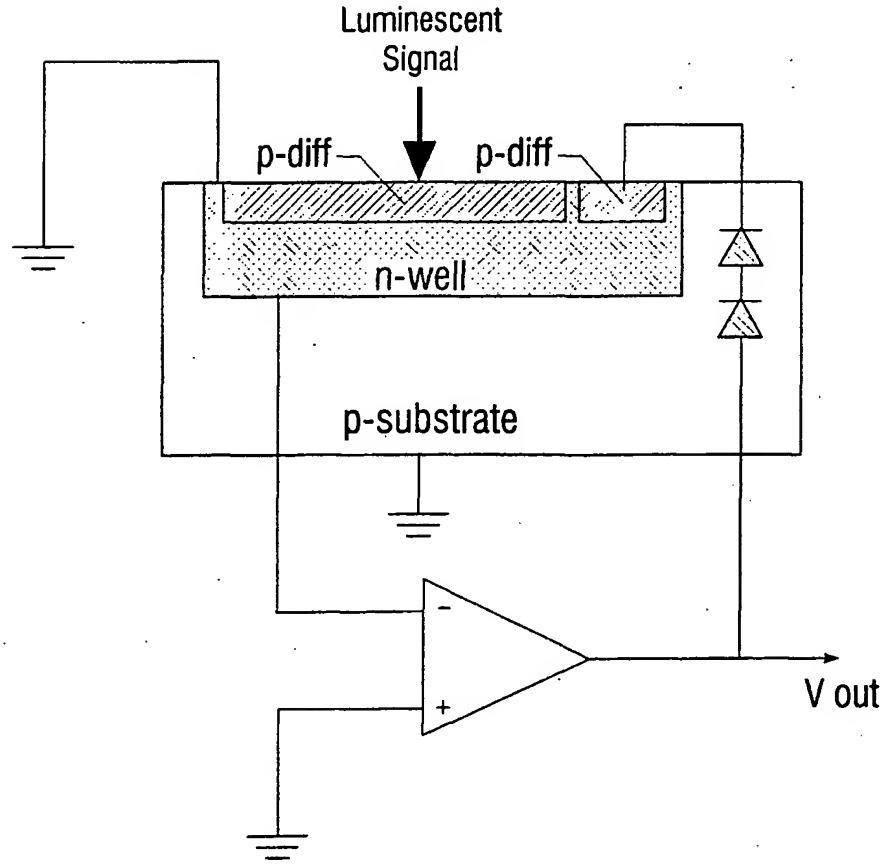
Luminescent
Signal
p-diff
p-diff
n-well
p-substrate
V out

FIG. 5B

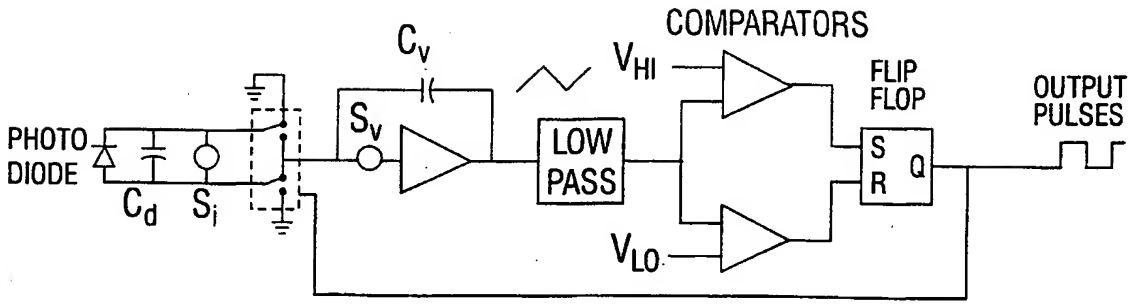
COMPARATORS
C_v
V_{HI}
FLIP
FLOP
OUTPUT
PULSES
PHOTO
DIODE
S_v
LOW
PASS
S
R
Q
C_d
S_i
V_{LO}

FIG. 5C

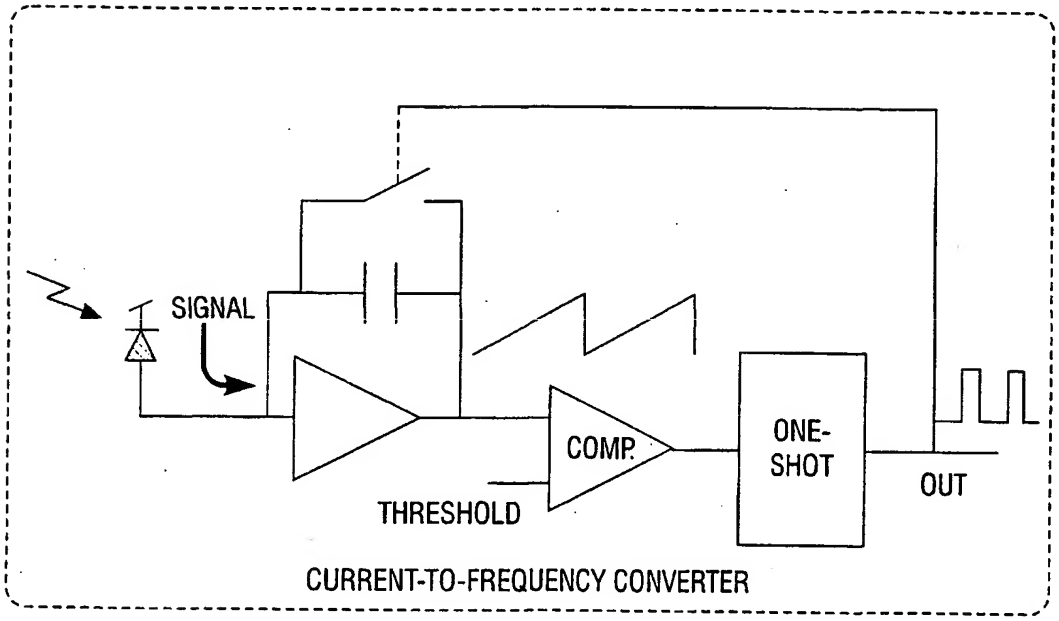
SIGNAL
COMP.
ONE-
SHOT
OUT
THRESHOLD
CURRENT-TO-FREQUENCY CONVERTER

FIG. 6

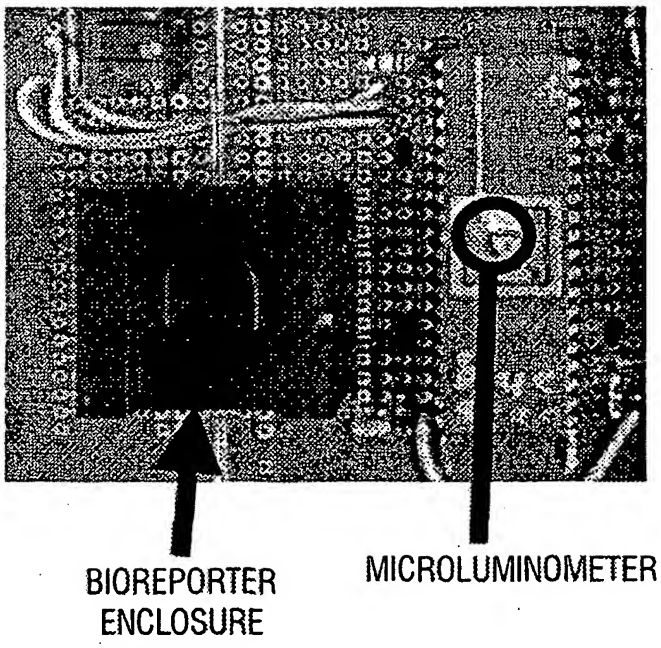
BIOREPORTER ENCLOSURE
MICROLUMINOMETER

FIG. 7

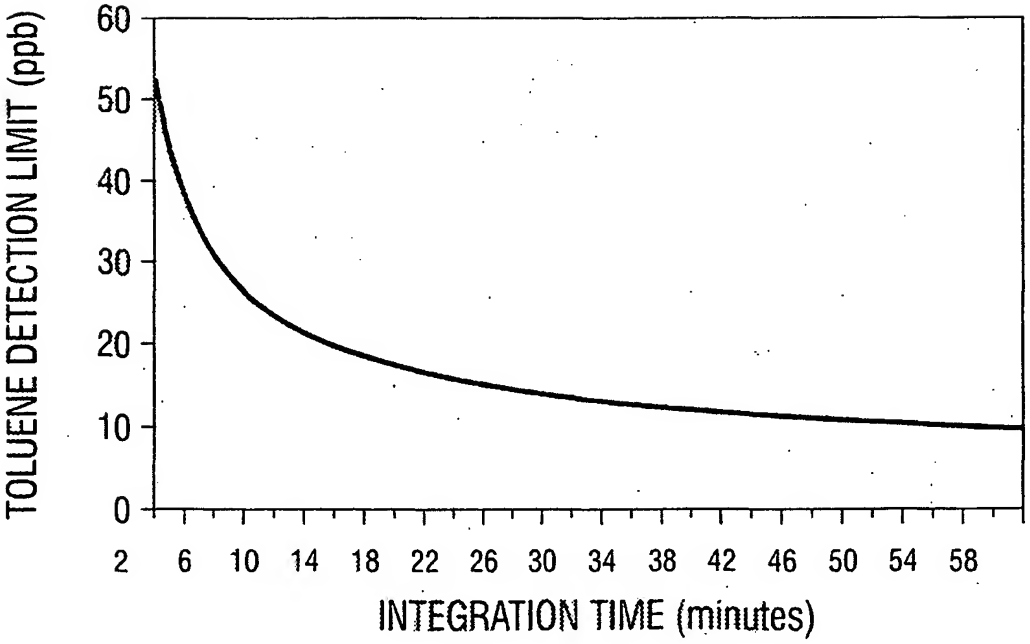
TOLUENE DETECTION LIMIT (ppb)
60
50
40
30
20
10
0
2 6 10 14 18 22 26 30 34 38 42 46 50 54 58
INTEGRATION TIME (minutes)

FIG. 8

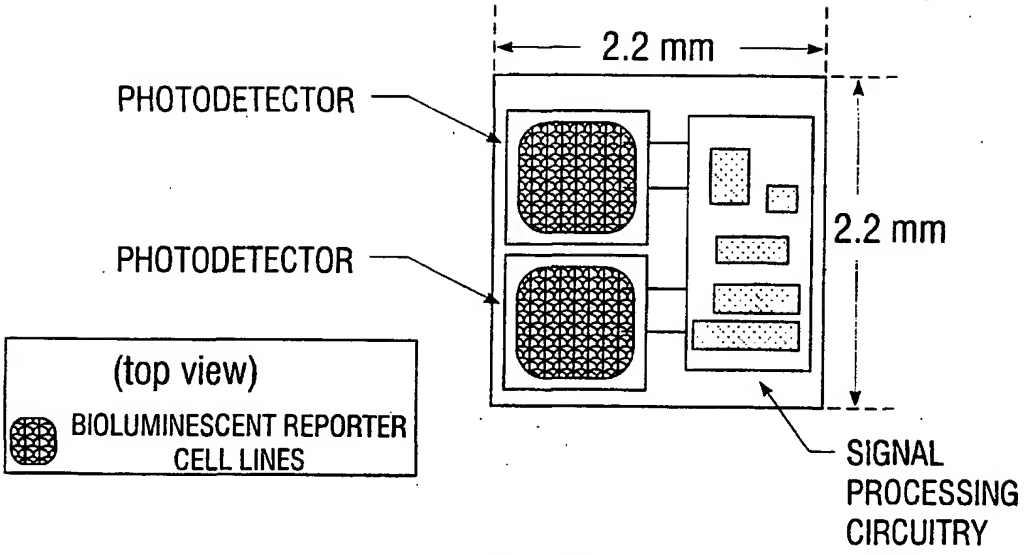
2.2 mm
PHOTODETECTOR
PHOTODETECTOR
2.2 mm
(top view)
BIOLUMINESCENT REPORTER CELL LINES
SIGNAL PROCESSING CIRCUITRY

FIG. 9A

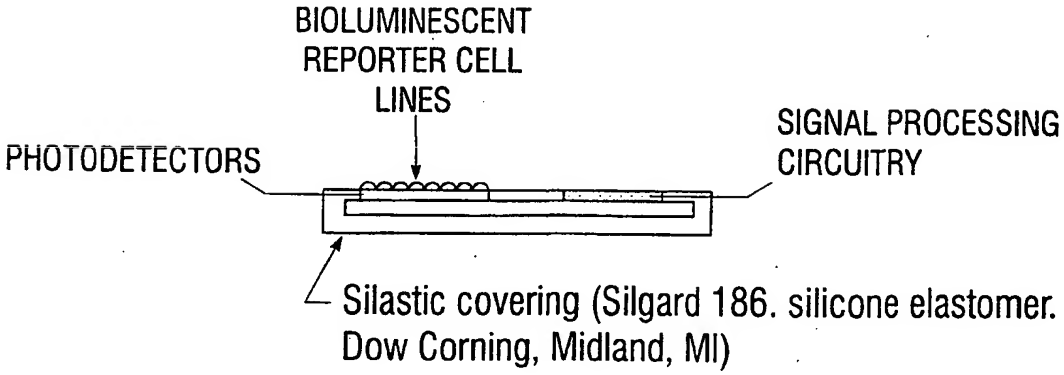
BIOLUMINESCENT REPORTER CELL LINES
PHOTODETECTORS
SIGNAL PROCESSING CIRCUITRY
Silastic covering (Silgard 186. silicone elastomer. Dow Corning, Midland, MI)

FIG. 9B

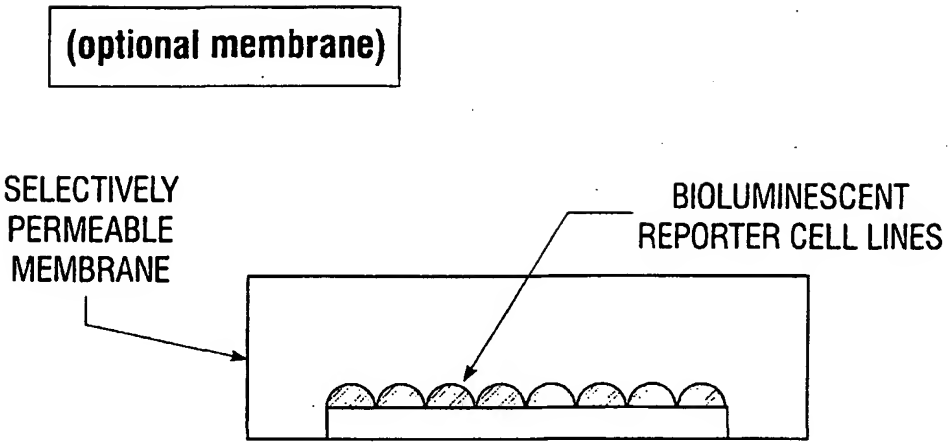
(optional membrane)
SELECTIVELY PERMEABLE MEMBRANE
BIOLUMINESCENT REPORTER CELL LINES

FIG. 9C

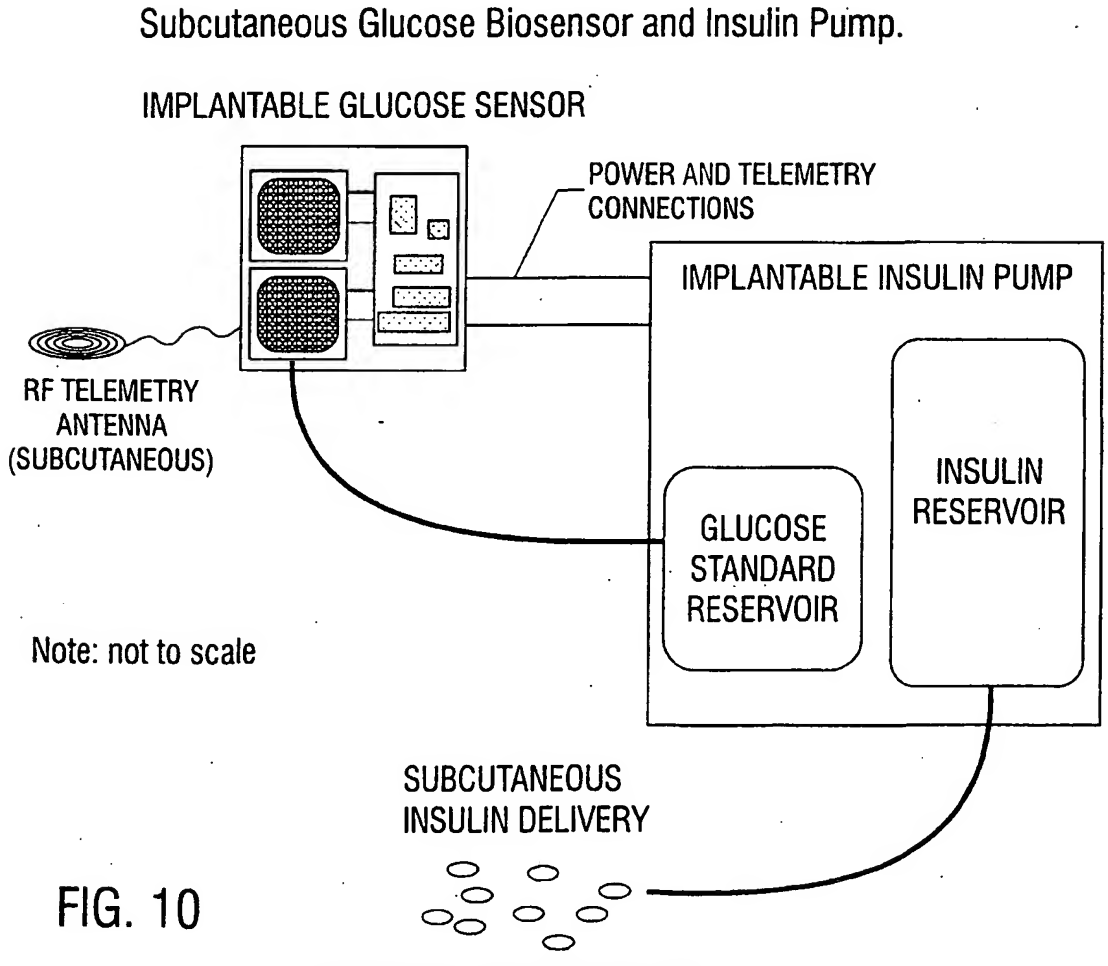
Subcutaneous Glucose Biosensor and Insulin Pump.
IMPLANTABLE GLUCOSE SENSOR
POWER AND TELEMETRY CONNECTIONS
IMPLANTABLE INSULIN PUMP
RF TELEMETRY ANTENNA (SUBCUTANEOUS)
GLUCOSE STANDARD RESERVOIR
INSULIN RESERVOIR
Note: not to scale
SUBCUTANEOUS INSULIN DELIVERY

FIG. 10

SEQUENCE LISTING

<110> SAYLER, GARY S.
SIMPSON, MICHAEL L.
APPLEGATE, BRUCE M.
RIPP, STEVEN A.

<120> IN VIVO BIOSENSOR APPARATUS AND METHOD OF USE

<130> 4300.004300

<140> UNKNOWN
<141> 1999-12-02

<150> 60/110,684
<151> 1998-12-02

<160> 7

<170> PatentIn Ver. 2.0

<210> 1
<211> 7669
<212> DNA
<213> Xenorhabdus luminescens

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<223> LUXC

<220>
<221> CDS
<222> (2671)..(3594)
<223> LUXD

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<222> (3776)..(4858)
<223> LUXA

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<221> CDS
<222> (4873)..(5847)
<223> LUXB

<220>
<221> CDS
<222> (6160)..(7272)
<223> LUXE

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ttttagtacg atagagccaa cattgagaaa ttatggggca acggaagcac aacttgaaga 120

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cgccagagcc aaaatacaca agcttaacca agaacagagg ttatacaaat gacagttaat 180

acagaggcac taataaacag cctaggcaag tcctaccaag aaatttttga tgaagggcta 240

attccttata ggaataagcc aagtggttct cctggggtgc ctaatatttg tattgacatg 300

gtgaaagagg ggatttttt gtcgtttgaa cggaatagta aaatattaaa cgaaattact 360

ttaagattgc ttagagacga taaagctttg tttatatttc caaatgaatt gccatcaccg 420

ttgaagcatt ctatggatag gggatgggtt agagaaaatt taggtgatct gattaaatca 480

ataccaccga gacaaatttt aaaaaggcag tttggttgga aagatctata tcgttttacg 540

gatgaaatca gtatgcagat ttcttatgat ttacgtgaac aggttaattc agtgactttc 600

ttgcttacat cagacgtgag ttggtaattt aatatatata cccttcatcc ttcaagttgc 660

tgctttgttg gctgctttct ctcaccccag tcacatagtt atctatgctc ctggggattc 720

gttcacttgc cgccgcgctg caacttgaaa tctattgggt atatgctatt ggtaattatg 780

gaaaattgcc tgatttatat ataacttaac ttgtaaacca gataataatt tacatgaata 840

ttatcacgta taaaaaaatt gcgattcttt taatttgaaa tagttcaatt taattgaaac 900

tttttattaa caaatcttgt tgatgtgaaa attttcgttt gctattttaa cagatattgt 960

taaacggaga aggcagcatg ttgatgattc actcagccag actgacagtt ttaagcggaa 1020

aattgcagag tatgatcgca ttctgataaa ggttacaggt cactcgcaac cagaatttca 1080

tctttgtata ttttgttttg ttatttacgt tgcagcaaga caaaaataga agaaacaaat 1140

atttatacaa cccgtttgca agagggttaa acagcaattt aagttgaaat tgccctatta 1200

aatggatggc aaat atg aac aaa aaa att tca ttc att att aac ggt cga   1250
               Met Asn Lys Lys Ile Ser Phe Ile Ile Asn Gly Arg
                 1               5                  10

gtt gaa ata ttt cct gaa agt gat gat tta gtg caa tcc att aat ttt   1298
Val Glu Ile Phe Pro Glu Ser Asp Asp Leu Val Gln Ser Ile Asn Phe
        15                  20                  25

ggt gat aat agt gtt cat ttg cca gta ttg aat gat tct caa gta aaa   1346
Gly Asp Asn Ser Val His Leu Pro Val Leu Asn Asp Ser Gln Val Lys
     30                  35                  40

aac att att gat tat aat gaa aat aat gaa ttg caa ttg cat aac att   1394
Asn Ile Ile Asp Tyr Asn Glu Asn Asn Glu Leu Gln Leu His Asn Ile
 45                  50                  55                  60

atc aac ttt ctc tat acg gta ggg caa cga tgg aaa aat gaa gaa tat   1442
Ile Asn Phe Leu Tyr Thr Val Gly Gln Arg Trp Lys Asn Glu Glu Tyr
                 65                  70                  75

tca aga cgc agg aca tat att cgt gat cta aaa aga tat atg gga tat   1490
```

```
Ser Arg Arg Arg Thr Tyr Ile Arg Asp Leu Lys Arg Tyr Met Gly Tyr
             80                  85                  90

tca gaa gaa atg gct aag cta gag gcc aac tgg ata tct atg att ttg   1538
Ser Glu Glu Met Ala Lys Leu Glu Ala Asn Trp Ile Ser Met Ile Leu
         95                 100                 105

tgc tct aaa ggt ggc ctt tat gat ctt gta aaa aat gaa ctt ggt tct   1586
Cys Ser Lys Gly Gly Leu Tyr Asp Leu Val Lys Asn Glu Leu Gly Ser
    110                 115                 120

cgc cat att atg gat gaa tgg cta cct cag gat gaa agt tat att aga   1634
Arg His Ile Met Asp Glu Trp Leu Pro Gln Asp Glu Ser Tyr Ile Arg
125                 130                 135                 140

gct ttt ccg aaa gga aaa tcc gta cat ctg ttg acg ggt aat gtg cca   1682
Ala Phe Pro Lys Gly Lys Ser Val His Leu Leu Thr Gly Asn Val Pro
                145                 150                 155

tta tct ggt gtg ctg tct ata ttg cgt gca att tta aca aag aat caa   1730
Leu Ser Gly Val Leu Ser Ile Leu Arg Ala Ile Leu Thr Lys Asn Gln
            160                 165                 170

tgc att ata aaa acc tca tca act gat cct ttt acc gct aat gca tta   1778
Cys Ile Ile Lys Thr Ser Ser Thr Asp Pro Phe Thr Ala Asn Ala Leu
        175                 180                 185

gcg cta agt ttt atc gat gtg gac cct cat cat ccg gta acg cgt tct   1826
Ala Leu Ser Phe Ile Asp Val Asp Pro His His Pro Val Thr Arg Ser
    190                 195                 200

ttg tca gtc gta tat tgg caa cat caa ggc gat ata tca ctc gca aaa   1874
Leu Ser Val Val Tyr Trp Gln His Gln Gly Asp Ile Ser Leu Ala Lys
205                 210                 215                 220

gag att atg caa cat gcg gat gtc gtt gtt gct tgg gga ggg gaa gat   1922
Glu Ile Met Gln His Ala Asp Val Val Val Ala Trp Gly Gly Glu Asp
                225                 230                 235

gcg att aat tgg gct gta aag cat gca cca ccc gat att gac gtg atg   1970
Ala Ile Asn Trp Ala Val Lys His Ala Pro Pro Asp Ile Asp Val Met
            240                 245                 250

aag ttt ggt cct aaa aag agt ttt tgt att att gat aac cct gtt gat   2018
Lys Phe Gly Pro Lys Lys Ser Phe Cys Ile Ile Asp Asn Pro Val Asp
        255                 260                 265

tta gta tcc gca gct aca ggg gcg gct cat gat gtt tgt ttt tac gat   2066
Leu Val Ser Ala Ala Thr Gly Ala Ala His Asp Val Cys Phe Tyr Asp
    270                 275                 280

cag caa gct tgt ttt tcc acc caa aat ata tat tac atg gga agt cat   2114
Gln Gln Ala Cys Phe Ser Thr Gln Asn Ile Tyr Tyr Met Gly Ser His
285                 290                 295                 300

tat gaa gag ttt aag cta gcg ttg ata gaa aaa ttg aac tta tat gcg   2162
Tyr Glu Glu Phe Lys Leu Ala Leu Ile Glu Lys Leu Asn Leu Tyr Ala
```

```
                305                 310                 315

cat ata tta cca aac acc aaa aaa gat ttt gat gaa aag gcg gcc tat   2210
His Ile Leu Pro Asn Thr Lys Lys Asp Phe Asp Glu Lys Ala Ala Tyr
            320                 325                 330

tcc tta gtt caa aaa gaa tgt tta ttt gct gga tta aaa gta gag gtt   2258
Ser Leu Val Gln Lys Glu Cys Leu Phe Ala Gly Leu Lys Val Glu Val
        335                 340                 345

gat gtt cat cag cgc tgg atg gtt att gag tca aat gcg ggt gta gaa   2306
Asp Val His Gln Arg Trp Met Val Ile Glu Ser Asn Ala Gly Val Glu
    350                 355                 360

cta aat caa cca ctt ggc aga tgt gtg tat ctt cat cac gtc gat aat   2354
Leu Asn Gln Pro Leu Gly Arg Cys Val Tyr Leu His His Val Asp Asn
365                 370                 375                 380

att gag caa ata ttg cct tat gtg cga aaa aat aaa acg caa acc ata   2402
Ile Glu Gln Ile Leu Pro Tyr Val Arg Lys Asn Lys Thr Gln Thr Ile
                385                 390                 395

tct gtt ttt cct tgg gag gcc gcg ctt aag tat cga gac tta tta gca   2450
Ser Val Phe Pro Trp Glu Ala Ala Leu Lys Tyr Arg Asp Leu Leu Ala
            400                 405                 410

tta aaa ggt gca gaa agg att gta gaa gca gga atg aat aat ata ttt   2498
Leu Lys Gly Ala Glu Arg Ile Val Glu Ala Gly Met Asn Asn Ile Phe
        415                 420                 425

cgg gtt ggt ggt gct cat gat gga atg aga cct tta caa cga ttg gtg   2546
Arg Val Gly Gly Ala His Asp Gly Met Arg Pro Leu Gln Arg Leu Val
    430                 435                 440

aca tat att tcc cat gaa aga cca tcc cac tat act gct aaa gat gtt   2594
Thr Tyr Ile Ser His Glu Arg Pro Ser His Tyr Thr Ala Lys Asp Val
445                 450                 455                 460

gcg gtc gaa ata gaa cag act cga ttc ctg gaa gaa gat aag ttc ctg   2642
Ala Val Glu Ile Glu Gln Thr Arg Phe Leu Glu Glu Asp Lys Phe Leu
                465                 470                 475

gta ttt gtc cca taa taggtaaaag aat atg gaa aat aaa tcc aga tat    2691
Val Phe Val Pro                 Met Glu Asn Lys Ser Arg Tyr
            480                             485

aaa acc atc gac cat gtt att tgt gtt gaa gaa aat aga aaa att cat   2739
Lys Thr Ile Asp His Val Ile Cys Val Glu Glu Asn Arg Lys Ile His
    490                 495                 500

gtc tgg gag acg ctg cca aaa gaa aat agt cca aag aga aaa aat acc   2787
Val Trp Glu Thr Leu Pro Lys Glu Asn Ser Pro Lys Arg Lys Asn Thr
505                 510                 515                 520

ctt att att gcg tcg ggt ttt gcc cgc agg atg gat cat ttt gcc ggt   2835
Leu Ile Ile Ala Ser Gly Phe Ala Arg Arg Met Asp His Phe Ala Gly
                525                 530                 535
```

```
ctg gca gag tat ttg tcg cag aat gga ttt cat gtg atc cgc tat gat   2883
Leu Ala Glu Tyr Leu Ser Gln Asn Gly Phe His Val Ile Arg Tyr Asp
            540                 545                 550

tct ctt cac cac gtt gga ttg agt tca ggg aca att gat gaa ttt aca   2931
Ser Leu His His Val Gly Leu Ser Ser Gly Thr Ile Asp Glu Phe Thr
        555                 560                 565

atg tcc ata gga aaa cag agt tta tta gca gtg gtt gat tgg tta aat   2979
Met Ser Ile Gly Lys Gln Ser Leu Leu Ala Val Val Asp Trp Leu Asn
    570                 575                 580

aca cga aaa ata aat aac ctc ggt atg ctg gct tca agc tta tct gcg   3027
Thr Arg Lys Ile Asn Asn Leu Gly Met Leu Ala Ser Ser Leu Ser Ala
585                 590                 595                 600

cgg ata gct tat gca agt cta tct gaa att aat gtc tcg ttt tta att   3075
Arg Ile Ala Tyr Ala Ser Leu Ser Glu Ile Asn Val Ser Phe Leu Ile
                605                 610                 615

acc gca gtc ggt gtg gtt aac tta aga tat act ctc gaa aga gct tta   3123
Thr Ala Val Gly Val Val Asn Leu Arg Tyr Thr Leu Glu Arg Ala Leu
            620                 625                 630

gga ttt gat tat ctc agc tta cct att gat gaa ttg cca gat aat tta   3171
Gly Phe Asp Tyr Leu Ser Leu Pro Ile Asp Glu Leu Pro Asp Asn Leu
        635                 640                 645

gat ttt gaa ggt cat aaa ttg ggt gct gag gtt ttt gcg aga gat tgc   3219
Asp Phe Glu Gly His Lys Leu Gly Ala Glu Val Phe Ala Arg Asp Cys
    650                 655                 660

ttt gat tct ggc tgg gaa gat tta act tct aca att aat agt atg atg   3267
Phe Asp Ser Gly Trp Glu Asp Leu Thr Ser Thr Ile Asn Ser Met Met
665                 670                 675                 680

cat ctt gat ata ccg ttt att gct ttt act gca aat aat gac gat tgg   3315
His Leu Asp Ile Pro Phe Ile Ala Phe Thr Ala Asn Asn Asp Asp Trp
                685                 690                 695

gta aag caa gat gaa gtt att aca tta cta tca agc atc cgt agt cat   3363
Val Lys Gln Asp Glu Val Ile Thr Leu Leu Ser Ser Ile Arg Ser His
            700                 705                 710

caa tgt aag ata tat tct tta cta gga agc tca cat gat ttg ggt gag   3411
Gln Cys Lys Ile Tyr Ser Leu Leu Gly Ser Ser His Asp Leu Gly Glu
        715                 720                 725

aac tta gtg gtc ctg cgc aat ttt tat caa tcg gtt acg aaa gcc gct   3459
Asn Leu Val Val Leu Arg Asn Phe Tyr Gln Ser Val Thr Lys Ala Ala
    730                 735                 740

atc gcg atg gat aat ggt tgt ctg gat att gat gtc gat att att gag   3507
Ile Ala Met Asp Asn Gly Cys Leu Asp Ile Asp Val Asp Ile Ile Glu
745                 750                 755                 760
```

```
ccg tca ttc gaa cat tta acc att gcg gca gtc aat gaa cgc cga atg   3555
Pro Ser Phe Glu His Leu Thr Ile Ala Ala Val Asn Glu Arg Arg Met
                765                 770                 775

aaa att gag att gaa aat caa gtg att tcg ctg tct taa aacctatacc   3604
Lys Ile Glu Ile Glu Asn Gln Val Ile Ser Leu Ser
            780                 785

aatagatttc gagttgcagc gcggcggcaa gtgaacgcat tcccaggagc atagataact 3664

ctgtgactgg ggtgcgtgaa agcagccaac aaagcagcaa cttgaaggat gaagggtata 3724

ttgggataga tagttaactc tatcactcaa atagaaatat aaggactctc t atg aaa  3781
                                                        Met Lys
                                                        790

ttt gga aac ttt ttg ctt aca tac caa ccc ccc caa ttt tct caa aca   3829
Phe Gly Asn Phe Leu Leu Thr Tyr Gln Pro Pro Gln Phe Ser Gln Thr
            795                 800                 805

gag gta atg aaa cgg ttg gtt aaa tta ggt cgc atc tct gag gaa tgc   3877
Glu Val Met Lys Arg Leu Val Lys Leu Gly Arg Ile Ser Glu Glu Cys
        810                 815                 820

ggt ttt gat acc gta tgg tta ctt gag cat cat ttc acg gag ttt ggt   3925
Gly Phe Asp Thr Val Trp Leu Leu Glu His His Phe Thr Glu Phe Gly
    825                 830                 835

ttg ctt ggt aac cct tat gtg gct gct gct tat tta ctt ggc gca acc   3973
Leu Leu Gly Asn Pro Tyr Val Ala Ala Ala Tyr Leu Leu Gly Ala Thr
840                 845                 850                 855

aag aaa ttg aat gta ggg act gcg gct att gtt ctc ccc acc gct cat   4021
Lys Lys Leu Asn Val Gly Thr Ala Ala Ile Val Leu Pro Thr Ala His
                860                 865                 870

cca gtt cgc cag ctt gaa gag gtg aat ttg ttg gat caa atg tca aaa   4069
Pro Val Arg Gln Leu Glu Glu Val Asn Leu Leu Asp Gln Met Ser Lys
            875                 880                 885

gga cga ttt cga ttt ggt att tgt cgg ggg ctt tac aat aaa gat ttt   4117
Gly Arg Phe Arg Phe Gly Ile Cys Arg Gly Leu Tyr Asn Lys Asp Phe
        890                 895                 900

cgc gta ttt ggc aca gat atg aat aac agt cgt gcc tta atg gag tgt   4165
Arg Val Phe Gly Thr Asp Met Asn Asn Ser Arg Ala Leu Met Glu Cys
    905                 910                 915

tgg tat aag ttg ata cga aat gga atg act gag gga tat atg gaa gct   4213
Trp Tyr Lys Leu Ile Arg Asn Gly Met Thr Glu Gly Tyr Met Glu Ala
920                 925                 930                 935

gac aac gaa cat att aag ttc cat aag gta aaa gtg ctg ccg acg gca   4261
Asp Asn Glu His Ile Lys Phe His Lys Val Lys Val Leu Pro Thr Ala
                940                 945                 950

tat agt caa ggt ggt gca cct att tat gtc gtt gct gaa tcc gct tcc   4309
```

```
Tyr Ser Gln Gly Gly Ala Pro Ile Tyr Val Val Ala Glu Ser Ala Ser
            955                 960                 965

acg act gaa tgg gcc gct caa cat ggt tta ccg atg att tta agt tgg   4357
Thr Thr Glu Trp Ala Ala Gln His Gly Leu Pro Met Ile Leu Ser Trp
        970                 975                 980

att ata aat act aac gaa aag aaa gca caa att gag ctt tat aac gag   4405
Ile Ile Asn Thr Asn Glu Lys Lys Ala Gln Ile Glu Leu Tyr Asn Glu
    985                 990                 995

gtc gct caa gaa tat gga cac gat att cat aat atc gac cat tgc tta   4453
Val Ala Gln Glu Tyr Gly His Asp Ile His Asn Ile Asp His Cys Leu
1000                1005                1010                1015

tca tat ata aca tcg gta gac cat gac tca atg aaa gcg aaa gaa att   4501
Ser Tyr Ile Thr Ser Val Asp His Asp Ser Met Lys Ala Lys Glu Ile
                1020                1025                1030

tgc cgg aat ttt ctg ggg cat tgg tat gat tcc tat gtt aat gcc aca   4549
Cys Arg Asn Phe Leu Gly His Trp Tyr Asp Ser Tyr Val Asn Ala Thr
            1035                1040                1045

acc att ttt gat gat tca gac aaa aca aag ggc tat gat ttc aat aaa   4597
Thr Ile Phe Asp Asp Ser Asp Lys Thr Lys Gly Tyr Asp Phe Asn Lys
        1050                1055                1060

gga caa tgg cgc gac ttt gtc tta aaa gga cat aaa aat act aat cgt   4645
Gly Gln Trp Arg Asp Phe Val Leu Lys Gly His Lys Asn Thr Asn Arg
    1065                1070                1075

cgc gtt gat tac agt tac gaa atc aat ccg gtg gga acc ccg cag gaa   4693
Arg Val Asp Tyr Ser Tyr Glu Ile Asn Pro Val Gly Thr Pro Gln Glu
1080                1085                1090                1095

tgt att gat ata att caa aca gac att gac gcc aca gga ata tca aat   4741
Cys Ile Asp Ile Ile Gln Thr Asp Ile Asp Ala Thr Gly Ile Ser Asn
                1100                1105                1110

att tgt tgt ggg ttt gaa gct aat gga aca gta gat gaa att atc tct   4789
Ile Cys Cys Gly Phe Glu Ala Asn Gly Thr Val Asp Glu Ile Ile Ser
            1115                1120                1125

tcc atg aag ctc ttc cag tct gat gta atg ccg ttt ctt aaa gaa aaa   4837
Ser Met Lys Leu Phe Gln Ser Asp Val Met Pro Phe Leu Lys Glu Lys
        1130                1135                1140

caa cgt tcg cta tta tat tag ctaaggaaaa tgaa atg aaa ttt ggc ttg   4887
Gln Arg Ser Leu Leu Tyr                     Met Lys Phe Gly Leu
    1145                1150                                1155

ttc ttc ctt aac ttt atc aat tca aca act att caa gag caa agt ata   4935
Phe Phe Leu Asn Phe Ile Asn Ser Thr Thr Ile Gln Glu Gln Ser Ile
                1160                1165                1170

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Ala Arg Met Gln Glu Ile Thr Glu Tyr Val Asp Lys Leu Asn Phe Glu
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cag att ttg gtg tgt gaa aat cat ttt tca gat aat ggt gtt gtc ggc   5031
Gln Ile Leu Val Cys Glu Asn His Phe Ser Asp Asn Gly Val Val Gly
       1190                1195                1200

gct cct ttg act gtt tct ggt ttt tta ctt ggc cta aca gaa aaa att   5079
Ala Pro Leu Thr Val Ser Gly Phe Leu Leu Gly Leu Thr Glu Lys Ile
   1205                1210                1215

aaa att ggt tca ttg aat cat gtc att aca act cat cat cct gtc cgc   5127
Lys Ile Gly Ser Leu Asn His Val Ile Thr Thr His His Pro Val Arg
1220                1225                1230                1235

ata gcg gaa gaa gcg tgc tta ttg gat cag tta agc gaa gga aga ttt   5175
Ile Ala Glu Glu Ala Cys Leu Leu Asp Gln Leu Ser Glu Gly Arg Phe
               1240                1245                1250

att tta gga ttt agt gat tgc gag aga aag gat gaa atg cat ttt ttc   5223
Ile Leu Gly Phe Ser Asp Cys Glu Arg Lys Asp Glu Met His Phe Phe
           1255                1260                1265

aat cgc cct gaa caa tac cag cag caa tta ttt gaa gaa tgc tat gac   5271
Asn Arg Pro Glu Gln Tyr Gln Gln Gln Leu Phe Glu Glu Cys Tyr Asp
       1270                1275                1280

att att aac gat gct tta aca aca ggc tat tgt aat cca aat ggc gat   5319
Ile Ile Asn Asp Ala Leu Thr Thr Gly Tyr Cys Asn Pro Asn Gly Asp
   1285                1290                1295

ttt tat aat ttc ccc aaa ata tcc gtg aat ccc cat gct tat acg caa   5367
Phe Tyr Asn Phe Pro Lys Ile Ser Val Asn Pro His Ala Tyr Thr Gln
1300                1305                1310                1315

aac ggg cct cgg aaa tat gta aca gca aca agt tgt cat gtt gtt gag   5415
Asn Gly Pro Arg Lys Tyr Val Thr Ala Thr Ser Cys His Val Val Glu
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tgg gct gct aaa aaa ggc att cct cta atc ttt aag tgg gat gat tcc   5463
Trp Ala Ala Lys Lys Gly Ile Pro Leu Ile Phe Lys Trp Asp Asp Ser
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aat gaa gtt aaa cat gaa tat gcg aaa aga tat caa gcc ata gca ggt   5511
Asn Glu Val Lys His Glu Tyr Ala Lys Arg Tyr Gln Ala Ile Ala Gly
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gaa tat ggt gtt gac ctg gca gag ata gat cat cag tta atg ata ttg   5559
Glu Tyr Gly Val Asp Leu Ala Glu Ile Asp His Gln Leu Met Ile Leu
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gtt aac tat agt gaa gac agt gag aaa gct aaa gag gaa acg cgt gca   5607
Val Asn Tyr Ser Glu Asp Ser Glu Lys Ala Lys Glu Glu Thr Arg Ala
1380                1385                1390                1395

ttt ata agt gat tat att ctt gca atg cac cct aat gaa aat ttc gaa   5655
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Lys Lys Leu Glu Glu Ile Ile Thr Glu Asn Ser Val Gly Asp Tyr Met
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gaa tgt aca act gcg gct aaa ttg gca atg gag aaa tgt ggt gca aaa   5751
Glu Cys Thr Thr Ala Ala Lys Leu Ala Met Glu Lys Cys Gly Ala Lys
       1430                1435                1440

ggt ata tta ttg tcc ttt gaa tca atg agt gat ttt aca cat caa ata   5799
Gly Ile Leu Leu Ser Phe Glu Ser Met Ser Asp Phe Thr His Gln Ile
   1445                1450                1455

aac gca att gat att gtc aat gat aat att aaa aag tat cac atg taa   5847
Asn Ala Ile Asp Ile Val Asn Asp Asn Ile Lys Lys Tyr His Met
1460                1465                1470                1475

tataccctat ggatttcaag gtgcatcgcg acggcaaggg agcgaatccc cgggagcata 5907

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ggtaaaacag gt atg act tca tat gtt gat aaa caa gaa atc aca gca agt   6198
              Met Thr Ser Tyr Val Asp Lys Gln Glu Ile Thr Ala Ser
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tca gaa att gat gat ttg att ttt tcg agt gat cca tta gtc tgg tct   6246
Ser Glu Ile Asp Asp Leu Ile Phe Ser Ser Asp Pro Leu Val Trp Ser
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tac gac gaa cag gaa aag att aga aaa aaa ctt gtg ctt gat gcg ttt   6294
Tyr Asp Glu Gln Glu Lys Ile Arg Lys Lys Leu Val Leu Asp Ala Phe
1505                1510                1515                1520

cgt cat cac tat aaa cat tgt caa gaa tac cgt cac tac tgt cag gca   6342
Arg His His Tyr Lys His Cys Gln Glu Tyr Arg His Tyr Cys Gln Ala
               1525                1530                1535

cat aaa gta gat gac aat att acg gaa att gat gat ata cct gta ttc   6390
His Lys Val Asp Asp Asn Ile Thr Glu Ile Asp Asp Ile Pro Val Phe
          1540                1545                1550

cca aca tca gtg ttt aag ttt act cgc tta tta act tct aat gag aac   6438
Pro Thr Ser Val Phe Lys Phe Thr Arg Leu Leu Thr Ser Asn Glu Asn
       1555                1560                1565

gaa att gaa agt tgg ttt acc agt agt ggc aca aat ggc tta aaa agt   6486
Glu Ile Glu Ser Trp Phe Thr Ser Ser Gly Thr Asn Gly Leu Lys Ser
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cag gta cca cgt gac aga cta agt att gag agg ctc tta ggc tct gta   6534
Gln Val Pro Arg Asp Arg Leu Ser Ile Glu Arg Leu Leu Gly Ser Val
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agt tat ggt atg aaa tat att ggt agt tgg ttc gat cat caa atg gaa   6582
Ser Tyr Gly Met Lys Tyr Ile Gly Ser Trp Phe Asp His Gln Met Glu
                1605                1610                1615

ttg gtc aac ctg gga cca gat aga ttt aat gct cat aat att tgg ttt   6630
Leu Val Asn Leu Gly Pro Asp Arg Phe Asn Ala His Asn Ile Trp Phe
            1620                1625                1630

aaa tat gtt atg agc ttg gta gag tta tta tat cct acg tca ttc acc   6678
Lys Tyr Val Met Ser Leu Val Glu Leu Leu Tyr Pro Thr Ser Phe Thr
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gta aca gaa gaa cac ata gat ttc gtt cag aca tta aat agt ctt gag   6726
Val Thr Glu Glu His Ile Asp Phe Val Gln Thr Leu Asn Ser Leu Glu
    1650                1655                1660

cga ata aaa cat caa ggg aaa gat att tgt ctt att ggt tcg cca tac   6774
Arg Ile Lys His Gln Gly Lys Asp Ile Cys Leu Ile Gly Ser Pro Tyr
1665                1670                1675                1680

ttt att tat ttg ctc tgc cgt tat atg aaa gat aaa aat atc tca ttt   6822
Phe Ile Tyr Leu Leu Cys Arg Tyr Met Lys Asp Lys Asn Ile Ser Phe
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tct gga gat aaa agt ctt tat att ata acg ggg gga ggc tgg aaa agt   6870
Ser Gly Asp Lys Ser Leu Tyr Ile Ile Thr Gly Gly Gly Trp Lys Ser
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tac gaa aaa gaa tct ttg aag cgt aat gat ttc aat cat ctt tta ttc   6918
Tyr Glu Lys Glu Ser Leu Lys Arg Asn Asp Phe Asn His Leu Leu Phe
        1715                1720                1725

gac act ttc aac ctc agt aat att aac cag atc cgt gat ata ttt aat   6966
Asp Thr Phe Asn Leu Ser Asn Ile Asn Gln Ile Arg Asp Ile Phe Asn
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caa gtt gaa ctc aac act tgt ttc ttt gag gat gaa atg caa cgt aaa   7014
Gln Val Glu Leu Asn Thr Cys Phe Phe Glu Asp Glu Met Gln Arg Lys
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cat gtt ccg ccg tgg gta tat gcg cga gca ctt gat cct gaa aca ttg   7062
His Val Pro Pro Trp Val Tyr Ala Arg Ala Leu Asp Pro Glu Thr Leu
                1765                1770                1775

aaa ccg gta cct gat ggg atg cct ggt ttg atg agt tat atg gat gca   7110
Lys Pro Val Pro Asp Gly Met Pro Gly Leu Met Ser Tyr Met Asp Ala
            1780                1785                1790

tca tca acg agt tat ccg gca ttt att gtt acc gat gat atc gga ata   7158
Ser Ser Thr Ser Tyr Pro Ala Phe Ile Val Thr Asp Asp Ile Gly Ile
        1795                1800                1805

att agc aga gaa tat ggt caa tat cct ggt gta ttg gtt gaa att tta   7206
Ile Ser Arg Glu Tyr Gly Gln Tyr Pro Gly Val Leu Val Glu Ile Leu
    1810                1815                1820
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cgt cgc gtt aat acg agg aaa caa aaa ggt tgt gct tta agc tta act   7254
Arg Arg Val Asn Thr Arg Lys Gln Lys Gly Cys Ala Leu Ser Leu Thr
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Glu Ala Phe Gly Ser
                1845

gctcatttgt tcaatgcttt tgcgaaacgt tttgtcgaac tctaggcgaa ggttctcgac 7362

tttccccgca tcaggggtat atacaagtaa aaaagctcag ggggtaaacc tgagcttggg 7422

atgttgattt ttaagtatga gatacatggg cggatttaaa taacggagtc agtttggaaa 7482

tatcaacggt cttttctgct ttatcgaggc tataagtttc ttgcagtttt aaccacaacc 7542

gcggagagct gccaagtact tgtgacagtt ttattgccat ctctggcgtg actgctgctt 7602

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Val His Leu Pro Val Leu Asn Asp Ser Gln Val Lys Asn Ile Ile Asp
         35                  40                  45

Tyr Asn Glu Asn Asn Glu Leu Gln Leu His Asn Ile Ile Asn Phe Leu
     50                  55                  60

Tyr Thr Val Gly Gln Arg Trp Lys Asn Glu Glu Tyr Ser Arg Arg Arg
 65                  70                  75                  80

Thr Tyr Ile Arg Asp Leu Lys Arg Tyr Met Gly Tyr Ser Glu Glu Met
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Ala Lys Leu Glu Ala Asn Trp Ile Ser Met Ile Leu Cys Ser Lys Gly
            100                 105                 110

Gly Leu Tyr Asp Leu Val Lys Asn Glu Leu Gly Ser Arg His Ile Met
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Asp Glu Trp Leu Pro Gln Asp Glu Ser Tyr Ile Arg Ala Phe Pro Lys
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Gly Lys Ser Val His Leu Leu Thr Gly Asn Val Pro Leu Ser Gly Val
145                 150                 155                 160

Leu Ser Ile Leu Arg Ala Ile Leu Thr Lys Asn Gln Cys Ile Ile Lys
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Thr Ser Ser Thr Asp Pro Phe Thr Ala Asn Ala Leu Ala Leu Ser Phe
            180                 185                 190

Ile Asp Val Asp Pro His His Pro Val Thr Arg Ser Leu Ser Val Val
        195                 200                 205

Tyr Trp Gln His Gln Gly Asp Ile Ser Leu Ala Lys Glu Ile Met Gln
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His Ala Asp Val Val Val Ala Trp Gly Gly Glu Asp Ala Ile Asn Trp
225                 230                 235                 240

Ala Val Lys His Ala Pro Pro Asp Ile Asp Val Met Lys Phe Gly Pro
                245                 250                 255

Lys Lys Ser Phe Cys Ile Ile Asp Asn Pro Val Asp Leu Val Ser Ala
            260                 265                 270

Ala Thr Gly Ala Ala His Asp Val Cys Phe Tyr Asp Gln Gln Ala Cys
        275                 280                 285

Phe Ser Thr Gln Asn Ile Tyr Tyr Met Gly Ser His Tyr Glu Glu Phe
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Lys Leu Ala Leu Ile Glu Lys Leu Asn Leu Tyr Ala His Ile Leu Pro
305                 310                 315                 320

Asn Thr Lys Lys Asp Phe Asp Glu Lys Ala Ala Tyr Ser Leu Val Gln
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Lys Glu Cys Leu Phe Ala Gly Leu Lys Val Glu Val Asp Val His Gln
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Leu Pro Tyr Val Arg Lys Asn Lys Thr Gln Thr Ile Ser Val Phe Pro
385                 390                 395                 400

Trp Glu Ala Ala Leu Lys Tyr Arg Asp Leu Leu Ala Leu Lys Gly Ala
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Glu Arg Ile Val Glu Ala Gly Met Asn Asn Ile Phe Arg Val Gly Gly
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Ala His Asp Gly Met Arg Pro Leu Gln Arg Leu Val Thr Tyr Ile Ser
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Arg Arg Met Asp His Phe Ala Gly Leu Ala Glu Tyr Leu Ser Gln Asn
50 55 60

Gly Phe His Val Ile Arg Tyr Asp Ser Leu His His Val Gly Leu Ser
65 70 75

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Leu Ala Val Val Asp Trp Leu Asn Thr Arg Lys Ile Asn Asn Leu Gly
100 105 110

Met Leu Ala Ser Ser Leu Ser Ala Arg Ile Ala Tyr Ala Ser Leu Ser
115 120 125

Glu Ile Asn Val Ser Phe Leu Ile Thr Ala Val Gly Val Val Asn Leu
130 135 140

Arg Tyr Thr Leu Glu Arg Ala Leu Gly Phe Asp Tyr Leu Ser Leu Pro
145 150 155

Ile Asp Glu Leu Pro Asp Asn Leu Asp Phe Glu Gly His Lys Leu Gly
60 165 170 175

Ala Glu Val Phe Ala Arg Asp Cys Phe Asp Ser Gly Trp Glu Asp Leu
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195 200 205

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225 230 235

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Tyr Gln Ser Val Thr Lys Ala Ala Ile Ala Met Asp Asn Gly Cys Leu
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Asp Ile Asp Val Asp Ile Ile Glu Pro Ser Phe Glu His Leu Thr Ile
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Phe Gly Leu Leu Gly Asn Pro Tyr Val Ala Ala Ala Tyr Leu Leu Gly
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Ala Thr Lys Lys Leu Asn Val Gly Thr Ala Ala Ile Val Leu Pro Thr
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Ala His Pro Val Arg Gln Leu Glu Glu Val Asn Leu Leu Asp Gln Met
                85                  90                  95

Ser Lys Gly Arg Phe Arg Phe Gly Ile Cys Arg Gly Leu Tyr Asn Lys
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Asp Phe Arg Val Phe Gly Thr Asp Met Asn Asn Ser Arg Ala Leu Met
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Glu Ala Asp Asn Glu His Ile Lys Phe His Lys Val Lys Val Leu Pro
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Thr Ala Tyr Ser Gln Gly Gly Ala Pro Ile Tyr Val Val Ala Glu Ser
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Ala Ser Thr Thr Glu Trp Ala Ala Gln His Gly Leu Pro Met Ile Leu
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Asn Glu Val Ala Gln Glu Tyr Gly His Asp Ile His Asn Ile Asp His
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Cys Leu Ser Tyr Ile Thr Ser Val Asp His Asp Ser Met Lys Ala Lys
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Glu Ile Cys Arg Asn Phe Leu Gly His Trp Tyr Asp Ser Tyr Val Asn
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Asp Lys Leu Asn Phe Glu Gln Ile Leu Val Cys Glu Asn His Phe Ser
            35                  40                  45

Asp Asn Gly Val Val Gly Ala Pro Leu Thr Val Ser Gly Phe Leu Leu
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Gly Leu Thr Glu Lys Ile Lys Ile Gly Ser Leu Asn His Val Ile Thr
     65                  70                  75
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Thr His His Pro Val Arg Ile Ala Glu Glu Ala Cys Leu Leu Asp Gln
 80                  85                  90                  95

Leu Ser Glu Gly Arg Phe Ile Leu Gly Phe Ser Asp Cys Glu Arg Lys
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Asp Glu Met His Phe Phe Asn Arg Pro Glu Gln Tyr Gln Gln Gln Leu
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Phe Glu Glu Cys Tyr Asp Ile Ile Asn Asp Ala Leu Thr Thr Gly Tyr
        130                 135                 140

Cys Asn Pro Asn Gly Asp Phe Tyr Asn Phe Pro Lys Ile Ser Val Asn
    145                 150                 155

Pro His Ala Tyr Thr Gln Asn Gly Pro Arg Lys Tyr Val Thr Ala Thr
160                 165                 170                 175

Ser Cys His Val Val Glu Trp Ala Ala Lys Lys Gly Ile Pro Leu Ile
                180                 185                 190

Phe Lys Trp Asp Asp Ser Asn Glu Val Lys His Glu Tyr Ala Lys Arg
            195                 200                 205

Tyr Gln Ala Ile Ala Gly Glu Tyr Gly Val Asp Leu Ala Glu Ile Asp
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His Gln Leu Met Ile Leu Val Asn Tyr Ser Glu Asp Ser Glu Lys Ala
    225                 230                 235

Lys Glu Glu Thr Arg Ala Phe Ile Ser Asp Tyr Ile Leu Ala Met His
240                 245                 250                 255

Pro Asn Glu Asn Phe Glu Lys Lys Leu Glu Glu Ile Ile Thr Glu Asn
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Ser Val Gly Asp Tyr Met Glu Cys Thr Thr Ala Ala Lys Leu Ala Met
            275                 280                 285

Glu Lys Cys Gly Ala Lys Gly Ile Leu Leu Ser Phe Glu Ser Met Ser
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Asp Asp Asn Ile Thr Glu Ile Asp Asp Ile Pro Val Phe Pro Thr Ser
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Val Phe Lys Phe Thr Arg Leu Leu Thr Ser Asn Glu Asn Glu Ile Glu
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Ser Trp Phe Thr Ser Ser Gly Thr Asn Gly Leu Lys Ser Gln Val Pro
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Arg Asp Arg Leu Ser Ile Glu Arg Leu Leu Gly Ser Val Ser Tyr Gly
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Met Ser Leu Val Glu Leu Leu Tyr Pro Thr Ser Phe Thr Val Thr Glu
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His Gln Gly Lys Asp Ile Cys Leu Ile Gly Ser Pro Tyr Phe Ile Tyr
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Leu Leu Cys Arg Tyr Met Lys Asp Lys Asn Ile Ser Phe Ser Gly Asp
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Lys Ser Leu Tyr Ile Ile Thr Gly Gly Gly Trp Lys Ser Tyr Glu Lys
225                 230                 235                 240

Glu Ser Leu Lys Arg Asn Asp Phe Asn His Leu Leu Phe Asp Thr Phe
                245                 250                 255

Asn Leu Ser Asn Ile Asn Gln Ile Arg Asp Ile Phe Asn Gln Val Glu
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Leu Asn Thr Cys Phe Phe Glu Asp Glu Met Gln Arg Lys His Val Pro
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Pro Trp Val Tyr Ala Arg Ala Leu Asp Pro Glu Thr Leu Lys Pro Val
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Gly Ser
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INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 99/28733

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N27/327 C12Q1/00 A61M5/142

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 756 351 A (ISACOFF EHUD Y ET AL) 26 May 1998 (1998-05-26) the whole document	1
A	WO 98 22820 A (MINIMED INC ;LAWRENCE LIVERMORE NATIONAL LA (US)) 28 May 1998 (1998-05-28) the whole document	1
A	US 5 109 850 A (BLANCO ERNESTO E ET AL) 5 May 1992 (1992-05-05) abstract	1
A	US 5 294 541 A (KAPLAN DAVID S ET AL) 15 March 1994 (1994-03-15) abstract	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 April 2000	20/04/2000

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